



**ELECTRON MICROSCOPY  
OF THE CARDIOVASCULAR SYSTEM**



REVISED AND ENLARGED ENGLISH EDITION

# ELECTRON MICROSCOPY OF THE CARDIOVASCULAR SYSTEM

*An Electron Microscopic Study with  
Applications to Physiology*

By

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To  
Dr GUSTAV J MARTIN

*who has steadfastly followed the progress of  
the present study with the greatest of under  
standing and helpfulness this volume is dedi  
cated in fond admiration*



## PREFACE

Over the past ten years the author of this volume has been occupied with electron microscopic studies of the ultra structure of the heart and blood vessels. The results of these studies have been published in various English, French, Italian and German scientific journals. A small summation of the author's results up to that time appeared in book form in New York in 1951 and a monograph in 1957 (1957g) in Germany. Since 1951 the methods of electron microscopic study, especially fixation and embedding of specimens, the use of plastic as an embedding medium and last but not least methods for obtaining ultra thin sections have been changed so completely that the present series of pictures made in continuation of the aforementioned studies are hardly comparable at all with those pictures published back in 1951. The photographs of recent years allow the recognition of previously unsuspected details, structures 100 Å or less in size being clearly visible. Thus we are now afforded a previously un hoped for widening of our horizons and an altogether new understanding of the ultra structure of tissues and their functions.

A principal prerequisite of all physiology is the recognition of the structure of organs and tissues if one wishes to understand their functions. This becomes especially apparent when one looks for example at the ultramicroscopic structure of capillaries and realizes that this structure is the keystone of countless physiological and pathological processes which cannot be understood without first understanding the exact make up of the capillary wall.

The specific cell type found in the lungs which was described by Schlotzer (1954) and independently by this author in 1955 on the basis of electron microscopic studies has now been well substantiated. It points to a new function of the lung unknown until now even though these cells are as yet not clearly understood as to their physiological significance.



In regard to the heart, electron microscopy has provided the answer or at least pointed to the final answer, to the age old question of why heart muscle can withstand a lifelong span of almost continuous work. It has also yielded new aspects for the mechanism of angina pectoris and of axon reflexes.

These few examples explain why, working in electron microscopy one comes to feel that the electron microscope will very soon be as vital to the future development of physiology pathology, and indeed every branch of biology, as the old light microscope was to the nineteenth century development of these sciences.

This little volume attempts to show in a limited area what the new method of electron microscopy has contributed thus far to the science of cardiology. The presentation is based almost wholly on the author's own research since, unfortunately his own pioneering work has to date, attracted but few co workers and associates in this field.

The technical difficulties of electron microscopy are very great even today and as always in histology one must face the question of just how much of the observed material is actual and natural structure and how much is artifact produced by technical inadequacies. The danger of the artifact which beguiles our judgment must not be underrated. On the other hand fear of misinterpretation must not be allowed to stand in the way of progress. To give an example from my own experience the great number of sarcosomes in the cardiac muscle of an animal when compared with its skeletal muscle (B Kisch 1950) could never have been an artifact, even if the pictures of the heart obtainable nine years ago were much less creditable than those taken today. The important advance to cardiology of recognizing the sarcosomes as vital parts of cardiac muscle fibers (B Kisch 1952) was achieved on the basis of those early and very poor pictures. The importance of sarcosomes to cardiology is of course being worked out more fully today by means of the much better pictures now available. Still today and even in the field of light microscopy artifacts play an important part. In the histology of the last 150 years they have led to many major discoveries. After all every stain is artificial and any method of fixation and embedding leads to falsified pictures.

An example of this from electron microscopy is the following increased periods of fixation in formalin, inadequate embedding in plastic and other such "mistakes" produce a rupture of the ultra microscopic webbing and holes between the organelles. This is of course undesirable and our technique must try to avoid such errors. However only a dull "artifactophobe" would discard such pictures as unusable failing to recognize that these very mistakes provide us with an ultramicroscopic teasing technique for the recognition of the finest organelles similar to the teasing techniques which proved so useful to light microscopy 100 years ago. Therefore purposely, some of these pictures were still incorporated in this book. Here as everywhere the advancement of science requires both good technique and a critical mind.

It must be emphasized that this volume still does not pretend to be a definitive monograph on a well-defined subject. Rather it is a summarizing presentation of work done to date a map with many blank areas of unexplored territory to serve as a statement of that which we hope already to comprehend and a pointer to the far greater area about which we know nothing and which, is yet remains to be explored.

The author has the pleasant duty of thanking those who helped him with the performance of these studies.

Above all he must thank the small group of the 1952 trustees of the American College of Cardiology (New York) who unanimously voted a large sum to buy an RCA electron microscope and to establish the Electron Microscope Research Institute then the greatest achievement of the American College of Cardiology solely to pursue the search for knowledge about the circulatory apparatus and its problems both theoretical and clinical. In this respect the Institute is still unique. The author had the honor of being entrusted with the directorship of this Institute now in possession of and sponsored by City Hospital at Elmhurst New York. For two years the National Heart Institute of the National Institutes of Health (Public Health Service) in Washington supported this research with a grant (H 1489R).

In the following three years the research was made possible partly through generous grants of the National Drug Company in Philadelphia. The awarding of these grants was primarily due

to the interest and understanding of the firm's Vice President and director of research, Dr. Gustav J. Martin.

Despite this help, however, the author was forced, lacking any other support, to personally bear the heavy burden of a great part of the research expenses. Without great personal sacrifices he would have been compelled to abandon these investigations many years ago. It was only in 1959 that he again received a grant from the National Institutes of Health (H-4094).

Special thanks also go to Brooklyn Medical Press in New York for generously permitting the use of pictures already published in the journal *Experimental Medicine and Surgery* and, last but not least, Charles C. Thomas, Publisher for prompt and careful execution of the printing of this work.

BRUNO KISCH

New York

# CONTENTS

	<i>Page</i>
<i>Preface</i>	vii
<i>Chapter</i>	
I Method of Investigation	3
II The General Structure of Muscle Fiber	10
III The Sarcolemm	14
IV The Minute Muscle Fibrils (Myofibril Muskelfibrille Myofibrille)	19
V The Protofibrils (Myofilament Protofibrillen Urfaser chen)	20
VI Ultramicroscopic Transverse Striations of Muscle Fibers	26
VII The Microscopic Cross Striation	28
VIII Pseudo-Striation of the Myofibrils	40
IX The Sarcosomes and the Sarcosome Theory of the Func tion of the Heart	46
A Nomenclature	46
B Location structure and function of the sarcosome	47
X Membranes and Other Structures Within the Cardiac Muscle Fiber	56
XI Intercalated Discs	91
XII The Nuclei	99
XIII Interstitial Cells	104
XIV The Endocardial Cells	108
XV The Conductive System (Purkinje Fibers)	112
XVI The Capillaries	119
A Normal capillaries	119
B The picture of the totally collapsed capillary	134
C Abnormal capillaries	137

XVII	Investigations on Cardiac Nerves and the Mechanism of Pain in Angina Pectoris	138
XVIII	The Mechanism of Aton Reflexes	147
XIX	Some Pictures From the Human Heart	153
XX	Final Remarks	162
XXI	References	165
	<i>Index</i>	177

**ELECTRON MICROSCOPY  
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## METHOD OF INVESTIGATION

Details of the construction and theory of the electron microscope will not be considered here. Readers interested in these details are referred to the many special and technical publications on the electron microscope. Readers interested only in a bird's-eye view of the inner workings of an electron microscope are referred to the excellent readable description by Bodo von Borries in *Gluckauf* (1955) and also to the article by the same author in *Rader Rundschau* (1956) or the book by Wyckoff (1949).

Basically, the electron microscope operates on the same principle of ray refraction as does the light microscope. Instead of refracting light rays, however, it refracts cathode rays consisting of free electrons.

The idea of substituting cathode rays for light rays, mainly the work of Bodo von Borries and E. Ruska, has radically changed the whole science of microscopy. Objects of previously invisible size were now brought into the sphere of our visual acquaintance. Light microscopy at its best had advanced the limits of visible objects to a size of about  $0.25 \mu$ . The ingenious mathematical and physical studies of Ernst Abbe and his painstaking research into all the materials that could be used in a light microscope as light refracting media paved the way for the triumphs achieved through light microscope research. Light microscopes, however, have a set finite limit which is determined not only by the instrument itself but by the wave length of the rays it employs. Abbe and other physicists demonstrated the fact that two separate particles can be seen as separate objects only when the distance between them is at least one half the wave length of the rays used to visualize them. This is the reason why microscopists have on



occasion substituted the shorter waved ultraviolet light for the ordinary white light. By doing this, they could increase somewhat the resolving power of the light microscope, and see some small otherwise invisible objects. Visible light has a wave length of approximately one half of one micron, and this determines the fact that the usual light microscopes have a maximal magnification power of only  $1500\times$ .

The magnifications possible when cathode rays are substituted for visible light rays are of a quite different order. Electrons radiating out from a piece of red hot metal can be directed at high speeds toward an anode in an electric field. According to von Borries such a beam of electrons, driven from a heated cathode toward an anode in an electric field of 50,000 volts achieves a speed approximately one half that of the speed of light. Electrons are the lightest particles known today. The wave length of these minute particles is only a tiny fraction (according to von Borries  $1/100,000$ ) of the wave length of visible light.

These basic facts made it at once apparent to the first pioneers of the idea of an electron microscope that here was a method whereby a new world existing, but as yet invisible, could at last be made visible to the eye. These expectations have already been realized to some small extent. Electron microscopes today manufactured by various firms the world over such as Siemens and Halske in Germany, Philips in Holland and RCA in the United States cannot of course use glass or quartz lenses to focus the electron beam. For this purpose special electric and magnetic lenses are used which act upon the electron beam in a high vacuum. It required ingenious research and the highest scientific exactitude to create machines which would meet the extremely demanding specifications necessary to make an actual functioning electron microscope of all the vague plans. The resulting machines are such extremely delicate and complicated precision instruments and so expensive (\$20,000 to \$30,000) that von Borries estimated that only about 1500 were in use throughout the world in 1955.

The reason that electron microscopy has as yet yielded so few returns to biology and medicine is not alone financial however. The technical difficulties confronting the electron microscopist are

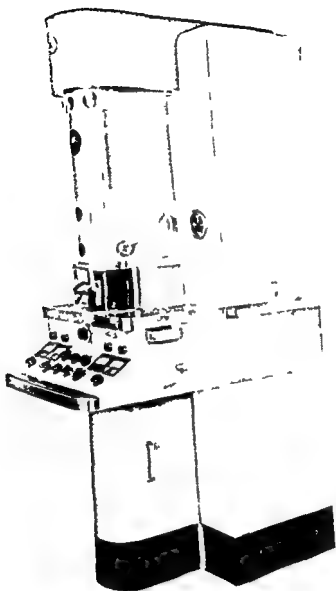


Figure 1 Exterior view of the RCA Universal Electron Microscope (Model AMU 2E) All the figures published in this volume were produced and photographed by means of a microscope of this type.

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The reason that electron microscopy has as yet yielded so few returns to biology and medicine is not alone financial however. The technical difficulties confronting the electron microscopist are

discouraging in scope. To begin with there is the question of proper specimen fixation preferably in the live state. Post mortem changes occur much more quickly in the size gradient viewed through an electron microscope than in the size gradient of the conventional light microscope. This is the reason that it is so hard for us to study the most vital organs such as the human heart in a state approximating life. Fifteen minutes postmortem in an unfixed state are sufficient to produce the grossest changes in the ultramicroscopic structure of the heart tissues. The human tissues (auricles & pectoralis) pictured in this volume were obtained unless otherwise noted at cardiac operations and the specimen was immediately fixed in 1% osmium tetroxide solution according to Palade's method (1952a). All animal tissues illustrated in this volume are likewise fixed in osmium tetroxide solution (0.1% up to 2%).

Despite its many virtues well known from light microscopy the osmium staining technique is certainly not the ultimate solution to the "staining" problems of ultramicroscopy. This author like many others has been experimenting along these lines and doesn't doubt that other "stains" will bring out clearly cell structures which are less well seen with the present osmium technique.

Another vital problem to electron microscopists is the thickness of specimen sections. Electron microscopic photography exposes a photographic plate by contact of the photographic emulsion with those particles of the electron beam which manage to pass through the specimen and are not deflected and absorbed by special screens. Just as the screen on a fluoroscope lights up at those places where the x rays strike it the screen of an electron microscope lights up where it is struck by the electron beam. On a photographic plate substituting for the screen consequently a black area arises at those places where the electrons strike the plate instead of the illuminating screen. Those parts of the specimen which do not permit passage of the electrons but scatter them to the absorbing screens appear as shadows on the observation screen and as light areas on the photographic plate. Thus the picture obtained with the electron microscope like the x ray plate represents a shadow image of the denser elements of the specimen. The more dense elements that there are standing

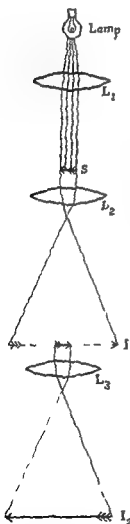
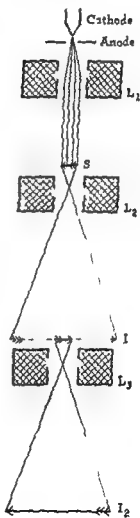
(a)  
Light  
Microscope(b)  
Electron  
Microscope

Figure 2. Comparative diagram of the beam within an electron microscope and within a light microscope. This and the previous figure have been made available through the courtesy of RCA.

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above or next to one another in the path of the electron beam the less usable for tissue studies is the resulting picture. It is therefore, apparent that the thicker the sections are the more this undesirable effect will be found. For this reason, the electron microscopic study of tissue sections was until very recently highly unsatisfactory. It was, therefore, thought preferable to split the specimen into dust like particles and make a suspension of these particles. An extremely thin film of this centrifuged suspension was next allowed to dry on a very fine membrane stretched over a metallic mesh and was sprayed with a molecular layer of some metal. Then the metal covered object was examined under the electron microscope.

For biology and medicine by and large such a method no matter how fruitful in its results could not be considered satisfactory. It was not histological at all rather it was a study of artificially produced tissue fragmentation. This method of necessity completely missed the significance of sarcosomes in muscle tissue. As soon as newer methods of sectioning allowed true tissue studies however, comparison of cardiac and skeletal muscles became possible and showed the different amount and the importance of sarcosomes clearly. Ingenious new microtomes now produce sections not in excess of  $0.2 \mu$  and often thinner than  $0.1 \mu$ . For specimens today  $0.1 \mu$  should be uniformly demanded although of course one must take into consideration here the nature of the specimen under investigation.

In order to obtain such thin sections one needs special microtomes and cutting knives (mostly glass but also diamonds). In addition the object must be embedded in a completely different way from that which was common in light microscopy. Even today much research is being done on this problem. The experiments range from attempts at freezing drying techniques (Peach and Baker 1949 Gesh 1956), to the techniques of embedding in plastic which are the ones most commonly used today (Borysko 1956).

Each one of the experimenters swears by his special method and the purely empirical decision as to which is the best remains to be decided by a comparison of the results in each technique.

In order to accustom himself to the unfamiliar order of magni

tude the reader is advised to consult the scale representing  $1 \mu$  ( $0.001 \text{ mm}$ ) for that particular magnification which is drawn on each of our pictures. Every millimeter on this scale represents a magnification of  $1000\times$ . For example if the scale line is  $20 \text{ mm}$  in length then the magnification of the object seen in the photograph is  $20000\times$ . At a magnification of  $50000\times$  which is not unusual to the electron microscope a  $1 \text{ mm}$  object would be  $50$  meters long (about  $150$  feet) which is approximately the height of a large size building.

We will not go into further technical details here. These details are contained in textbooks and in various specialized publications. It should be clear after reading this chapter however why the use of electron microscopy is a research tool by medicine and biology is still in its infancy.

The following orders of magnitude which appear repeatedly in the text and illustrations are freshed here for the reader

$$1 \text{ inch} = 25 \text{ mm}$$

$$1 \text{ mm} = 1000 \mu (\mu = \text{micron})$$

$$1 \mu = 10000 \text{ \AA } (\text{\AA} = \text{angstrom})$$

$$1 \text{ mm} = 1000000 \text{ \AA }$$



## II.

### THE GENERAL STRUCTURE OF MUSCLE FIBER

Basically, the structure of the muscle fiber of cardiac muscle is very similar to the muscle fiber of skeletal muscle. However, the two structures differ widely in several ways.

Cardiac muscle fibers like skeletal muscle fibers are surrounded by a membrane which can be clearly demonstrated in the electron microscope. The structure of the membrane and its characteristics will be discussed more fully below. The membrane surrounding cardiac muscle fibers is simpler in its structure than the sarcolem of skeletal muscle. However in the following text, this membrane will always be referred to as the sarcolem of the cardiac muscle fiber. The sarcolem surrounds all the primitive contractile elements contained in one muscle fiber that is the myofibrils, which have also been referred to as the primitive fibrils. Large numbers of these myofibrils are surrounded by a single sarcolem. They represent the simplest independent contractile element of the muscle (see diagram Fig 3). Even in electron microscope pictures of good quality these little fibrils appear as separated structures and at the beginning of our research their appearance caused me to call them bamboo stick fibrils. Since these primitive fibrils appear as clearly separated structures within the muscle fiber I see no reason why the heart muscle should be considered as a syncytium despite the fact that in heart muscle in contrast to the flight muscle of insects a parting of the myofibrils is more often seen. After all the spinal nerves are not looked upon as a syncytium though they arise by a joining of two nerve roots because the individual nerve fibers are entirely separated from one another. I was not able to discover a separate membrane surrounding the individual myofibrils. This is in

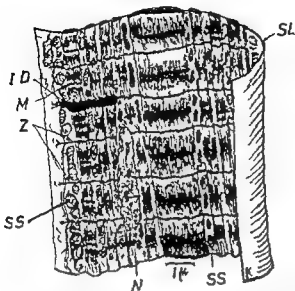


Figure 3 Schematic diagram of an uncoded cardiac muscle fiber - The individual myofibrils whose striations can be seen are connected by means of Z bands SL-sarcolemm SS-sarcosome \-nucleus ID-intercalated disc M-middle line

agreement with the work of Hodge Huxley and Spiro (1954) and that of Chapman (1954) However the outer surface of the myofibril presents an interphase in relation to the surrounding sarcoplasm which may fall into the biological category of a membrane

The only connection between the primitive fibers or myofibrils of the heart is in the form of lamellae or band like tiny threads which in the region of the Z-zone stretch from one myofibril to the other These tiny threads the Z bands are not identical with the Z zone of the myofibril even though when present they are always found in this region of the myofibril They are clearly demonstrable in the heart especially in ultramicroscopic teasing preparations (see Figs 4 and 25) We are not certain whether these bands are also always present in striated skeletal muscle Pease and Baker (1949) have seen them in certain striated muscles However we have been able to show with certainty that in selected skeletal muscles for example the flight muscles of

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Each of the above organelles of the cardiac muscle fiber will be discussed separately below and illustrated with pictures. Each of them is found within or at the border of the heart muscle fiber. Between the individual muscle fibers one also finds cells and fibers of which part belong to the connective tissue. Of special significance are the blood vessels and lymphatic vessels found there. As yet we have not had time to thoroughly investigate all of these structures found in the spaces between cardiac muscle fibers and therefore of the whole group mainly the ultra microscopic picture of the capillary and the intracardiac nerves will be presented here and also some interstitial cells since our experience in this direction is somewhat greater.

certain insects, the Z bands are not present (see Kisch, 1955 1959a, Kisch and Philpott 1955, the reader is also referred to Figures 21, 27, and 27a) More will be said in later chapters about the Z bands Z zones and the zones surrounding Z zones (PZ) Our latest investigations seem to prove the absence of the Z bands, but presence of Z zones also in myofibrils of the conductive system of the heart (see below)

A further, regular component of cardiac muscle is the nucleus with its enclosed nucleoli Of great significance also are the small bodies, already seen early in the last century by Bowman, Henle and Koelliker, which until 1950 received no attention from either physiologists or cardiologists, though at the turn of the century such prominent anatomists as G Retzius and Holmgren had shown interest in these organelles Despite the indifference shown them these little bodies are vital components of the heart muscle (B Kisch 1952a 1952b 1957g), they are the sarcosomes The name sarcosome was applied to the little bodies in 1890 by G Retzius who observed them mainly in the flight muscles of dragonflies They are also present in varying lesser numbers in skeletal muscles and their abundance depends on the type of the muscle in question (see Kisch 1951 1956f) They are found in countless numbers in the heart muscles of all animals which we have so far investigated (mammals man fish, frog)

Finally, one other structure must be mentioned It is a very fine membrane which has been found occasionally within the heart muscle fiber and whose physiological significance is not yet understood Some of these structures may perhaps be part of the endoplasmic reticulum which will be discussed later or belong to that system which earlier histologists called Ergastoplasm

We would also like to mention smallest droplets present within the muscle fiber in the cytoplasm around the myofibrils They are apparently not part of the endoplasmic reticulum Their diameter is about 300-1000 Å They serve probably in the exchange of material between muscle fiber and the intercellular space much as do similar droplets in the capillary wall and in nerve axons (see Fig 78) I have called this important procedure droplet transfer or droplet dialysis in contradistinction to the well known molecular dialysis (Kisch 1960)



Figure 4 Muscle fibers from the aorta of an albino mouse. SL—sarcolemma. It should be noted that the Z bands connect the individual myofibrils and are connected with the sarcolemma at the place where it is indented. The numerous sarcosomes and mitochondria of varying sizes and form are all found within the muscle fiber partly between the myofibrils, partly between the sarcolemma and the myofibrils and partly in proximity to the nucleus (N). Arrows indicate sarcosomes near the nucleus. 12,000X.

### III.

## THE SARCOLEM

The ultramicroscopic structure of the sarcolem of the skeletal muscle of insects and mammals has been repeatedly described and pictured in recent years (Draper and Hodge, 1949, B Kisch, 1951, 1955c, Bennet and Porter 1953 H Ruska, 1954 etc )

The muscle fibers of the hearts of all vertebrates investigated thus far, including humans, show a thin encompassing membrane which in the electron microscope appears as a double boundary line approximately 200 Å to 300 Å in thickness. The membrane appears free of all connective tissue and therefore it is more delicate than the sarcolem of skeletal muscle. The membrane has been described by Draper and Hodge (1949) B Kisch (1951 1957g) and others

In some hearts as we were able to show groups of thin muscle fibers together with their sarcolems are grouped into larger bundles of fibers by means of a further membrane extending from cells having definite nuclei. Thus we could differentiate a true sarcolem as described above from this other more variable membrane (B Kisch 1954 1957g). This other membrane is not a simple sarcolem. Rather it has the appearance of cells lying between the muscle fibers having clearly visible nuclei and which enclose by means of their protoplasm a group of muscle fibers together with their sarcolems (see Figs 5 and 6). We shall return to this subject in a later chapter. The designation sarcolem of the second order should not be construed to mean that these membranes are of the same sort or of the same origin as the true sarcolems of the separate cardiac muscle fibers which have no nuclei of their own. Rather these second order sarcolems are made up of cells whose cytoplasm encloses groups of muscle fibers in pipe like fashion (Fig 6). I would further hesitate to



Figure 6 Cross section through muscle fibers of the auricle of an albino mouse. Here as in the fish heart (Figure 5) one sees muscle fibers with a true sarcolem (SL) bound together by a "sarcolem of the second order" the latter being made up of the cytoplasm of a neighboring interstitial cell which surrounds the muscle fibers in the manner of a pipe. Several different types of plasmasomes are seen within its cytoplasm. 12,000x





Figure 5 From the ventricle of a pipefish. A number of cross sectioned muscle fibers whose true sarcolem (SL 1) is recognizable are enclosed membrane like by a second order sarcolem (SL-2). This second order sarcolem is made up of wing shaped extensions of cytoplasm of cells whose nuclei (N) are easily recognized. Within the muscle fiber one can see the ribbon shaped perpendicularly sectioned myofibrils (MF) and the smaller rounder sarcosomes (SS). 7000X

## IV

### THE MINUTE MUSCLE FIBRILS (MYOFIBRIL, MUSKELFIBRILLE, MYOFIBRILLE)

The muscle fibril like the muscle fiber has a double striation axial and transverse. The transverse striation (running parallel to the transverse diameter) is represented by the bands and zones known to us from light microscopy which are responsible for all of the cross striation of striated musculature. The axial striations which run parallel to the long axis of the muscle fibril are by virtue of the structure of the fibril made up of the finest of structures the arch fibers or protofibrils. In this respect no major difference can be demonstrated between the myofibrils of skeletal musculature and those of cardiac muscle. As our pictures illustrate both show basically the same structure.

The width of the individual myofibrils varies considerably in the heart of different animals (see Fig 3). For the rabbits ventricle the average width of myofibrils is  $0.86\mu$  (range  $0.2\mu-2.2\mu$ ). For the cow it is  $1.82\mu$  (range  $0.4\mu-5.2\mu$ ). For the human ventricle the value is approximately between these two but there are no values available as yet for the normal human heart.

designate this structure as being similar to the perimysium (Bruno Kisch 1954, 1957g, recently, Fawcett and Selby, 1958)

It is typical of the first order or true sarcolem of the individual muscle fibers both in the ventricle and in the auncle that when it is cut lengthwise, it usually has the appearance of a garland (see Figs 4, and 23) The indentations of this garland are often visibly attached to the Z bands, which bind together the myofibrils Frequently, it is very easy to recognize this attachment (see Figs 4 23 and 24) We shall discuss this more fully later This appearance had already been described in regard to skeletal muscle by light microscopists and electron microscopists have since confirmed the observation in part (Perse and Baker, 1949) It definitely occurs in heart muscle (Kisch 1951 1957g and more recently Poche and Lindner 1955 Lindner 1957) The indentations are always found in the region of the Z band even when the attachment to the indentation of the sarcolem cannot be clearly demonstrated All this is also true of the human heart (see Figures 23 and 24) In the pockets of the sarcolem created by the above described outpouchings one often finds one or more sarcosomes lodged between the sarcolem and the myofibrils (B Kisch 1951 1957g) In auricular muscle fibers one frequently finds numerous sarcosomes in these sarcolem pockets (B Kisch, 1956a, b and f) Less frequently the same is true in muscle fibers from the ventricle (see Figs 4 and 31)

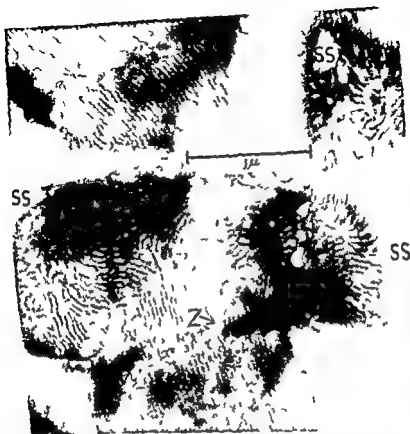


Figure 7 Sarcosomes and myofibrils from the flight muscle of a wasp. In this figure the protofibrils and the lamellar structure of the interior of the sarcosomes is clearly demonstrated as is the fact that the protofibrils traverse the Z zone. The distance between the center of one protofibril and the center of the neighboring one is approximately 300 to 350 Angstroms. Notice also the little vacuoles in the sarcosomes (SS). 34,000 $\times$  (From Kusch and Philpott, 1953)

being held together only in the region of the Z band. A different picture results if a muscle fibril instead of being sectioned along the longitudinal axis is sectioned obliquely or transversely. The protofibrils are then seen as parallel lines or frequently as parallel dotted lines in which each dot represents a protofibril which has been cut across obliquely. In cross section the protofibrils appear as regular dots packed in a hexagonal pattern. The distance be

## THE PROTOFIBRILS (MYOFILAMENT, PROTOFIBRILLEN, URFASERCHEN)

In longitudinal sections of a muscle fibril, one can recognize the protofibrils as striations running parallel to the long axis of the fibril. The distance between the center of two of these striations is approximately 250 to 350 Å and the diameter of a protofibril is approximately half of this distance. Bennet and Porter (1953) have stated that the diameter of a protofibril of the breast muscle of a chicken is approximately 150 Å, while Hodge (1955) gave the same diameter in the muscles of insects as approximately 120 Å and in mammals and amphibians as 100–130 Å (Hodge Huxley and Spiro 1954). In my own investigations I found on the average the following measurements for the protofibrils of the heart: 150 Å in the heart of a bat (1954), 200 Å in the heart of the guinea pig (1955), approximately 150 Å in the ventricular musculature of the human heart (see Figs 7, 8, 9, 10), and approximately 215 Å in the human pectoralis muscle. Philpott (1954) states that the diameter of the protofibril of the flight muscle of the wasp is  $220 \pm 15$  Å while Chapman (1954) gives the diameter in the same tissue as 100 Å. The diameter of the protofibrils in the heart of mammals has been found to be 100–150 Å by Moore and Ruska (1957) and that of the rat 110 Å (Porter and Palade 1957). In the wing muscle of the meat fly I recently found the center to center distance of the individual protofibrils circa 350 Å. That would mean a protofibril diameter of circa 170 Å. Occasionally, if the specimen has not been properly fixed and therefore, the tissue structures have split apart one finds on examining these ultra microscopic teasing preparation that the fibrils have been split up into bundles of protofibrils the bundles

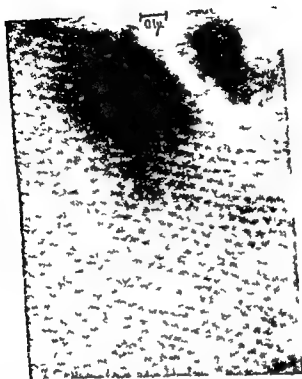


Figure 9 Cross sectioned myofibril of the same human heart in bigger enlargement From Kisch Cavusoglu and Marangoni (1959) 70 000 $\times$

the mammalian heart the distance is about 300 Å (see Figs 8 and 10). I have observed hexagonal packing in the cardiac muscle of the human auricle as well. Hodge Hurley and Spiro (1954) and Hodge (1956) also mention this particular type of organization of the protofibrils of the muscle fibers in the heart. I found the distance between two protofibrils as measured from center to center in the guinea pig and in the rat heart muscle sectioned lengthwise to be approximately 300 to 400 Å and in recent investigations I found the center to center distance of the protofibrils in cross sectioned myofibrils in the human heart circa 300 to 340 Å and in the flight muscle of the humble bee circa 300 to 350 Å (see Figs 7, 8 and 9).

The hexagonal packing of the protofibrils of the muscle fibril reminds one strongly of the latticed structure of crystals. I have



Figure 8 : Cross section of a myofibril of the human ventricle showing the hexagonal packing of the cross sectioned protofibrils. The big, dark bodies are sarcosomes. 40 000 $\times$

tween such dots is as follows: in insect muscles approximately 300 Å (Chipmann 1954; Hodge 1955); in mammals and amphibians 200 to 300 Å (Hodge, Huxley, Spiro 1954); in guinea pig heart approximately 300 Å (according to my own findings). Moore and Ruskai (1957) found it to be 280 to 330 Å in the heart of mammals. The above mentioned hexagonal packing of the protofibrils, which we show here in our pictures of heart muscle, has already been repeatedly shown in the striated skeletal muscles of both mammals and insects (first in 1950 by Morgan and his co-workers and more recently with a series of excellent pictures in insect muscles by Hodge (1955, 1956)). In specimens of insect muscles Hodge (1955) estimates the distance between protofibrils to be 600 Å. As previously mentioned in our pictures of

Philpott (1955) in the flight muscle of the bumble bee and likewise by Hodge in insect muscle (1955). The protofibrils appear as thickened and darker in the M and in the Z zone (see Fig. 7) in mammals as well as in the human heart. In vertebrate musculature a similar occurrence has been shown in metal shaded preparations.

TABLE I  
DIAMETER OF MYOFILAMENT

Specimen	Animal	$\lambda$	Author
1 Wing muscle	Insect	120	Hodge 1955
2 Wing muscle	Veal fib	170	Bruno Kisch 1954 1959
3 Flight muscle	Wasp	20	Philpott 1954
4 Flight muscle	Wasp	100	Chipman 1954
5 Breast muscle	Chicken	150	Bennet Porter 1953
6 Muscle	Mammals	100-130	Hodge Huxley Spiro 1954
	Amphibia		
7 Pectoralis muscle	Man	215	Bruno Kisch 1954 1959
8 Heart	Bat	150	Bruno Kisch 1954 1959
9 Heart	Guinea pig	up to 200	Bruno Kisch 1954 1959
10 Heart	Man	150	Bruno Kisch 1954 1959
11 Heart	Mammal	100-150	Moore and Ruska 1957
12 Heart	Rat	110	Porter and Palade 1957
13 Heart	Do Guinea pig	120	Lindner 1957
	Frog		
14 Heart	Cow	ca 150	Bruno Kisch 1959

TABLE II  
CENTER TO CENTER DISTANCE OF MYOFILAMENT

Specimen	Animal	$\lambda$	Author
1 Wing muscle	Insect		Chipman 1954
		300	Hodge 1955
2 Muscle	Insect	600	Hodge 1955
3 Wing muscle	Bumble bee	300-350	Bruno Kisch
4 Muscle	Mammal	200-300	Hodge Huxley Spiro 1958
	Amphibia		
5 Heart	Guinea pig	300	Bruno Kisch 1954
6 Heart	Rabbit	300	Bruno Kisch
7 Heart	Rat	300-400	Bruno Kisch
8 Heart	Man	300-340	Bruno Kisch
9 Heart	Mammal	280-300	Moore and Ruska, 1957
10 Heart	Cow	350-400	Bruno Kisch 1959
11 Wing muscle	Honey bee	300	Bruno Kisch



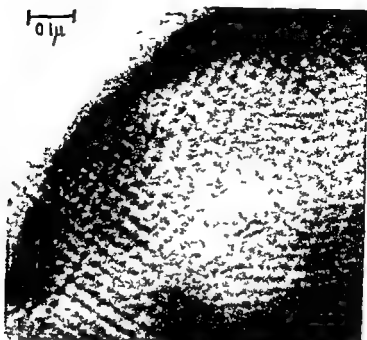


Figure 10 Cross section of myofibril of the wing muscle of a bumble bee showing the protofibrils in hexagonal packing. 100 000×

repeatedly pointed out the fact (1951a, 1954b, etc.) that the requirements demanded of a crystal—namely, homogeneity of the tiniest similar component parts among each other, double refraction of a light beam, and the existence of an optical axis—can also be applied exactly to skeletal muscle and cardiac muscle and that furthermore the physical properties of muscles are such that one should consider including them under the classification of Otto Lehmann's liquid crystals. Further studies are still urgently needed here.

It would seem that the protofibrils pass without interruption through the various zones of striation of cardiac muscle (Hodge 1956) as can be seen especially well in ultramicroscopic teasing preparations. This has been shown by various authors to be the case in the skeletal muscles of vertebrates and in the flight muscles of insects (see Hall, Jakus and Schmitt 1946; Rozsa, Szent-Gyorgyi and Wyckoff 1950; Bennet and Porter 1953; Chapman 1954; Hodge, 1956). A clear example of the fact that the protofibrils also pass through the Z zone was pointed out by Kisch and



Figure 11. Myofibrils from the ventricle of a bat. Sarcosomes are visible between the myofibrils (SS). The ultramicroscopic cross striation is clearly visible occurring at a spacing of approximately 300 to 350 Å. 40,000X.

It seems therefore that ultramicroscopic rhythmicity of the muscle fibril occurs as universally in the cardiac muscle of vertebrates as according to Hodge (1949, 1956) it does in their skeletal muscles.

The measurements of ultramicroscopic and microscopic structures as given here and in the following chapter are summarized in Tables I and II. They depend unquestionably not only on the functional stage of the tissue at the moment of fixating but also on the different technical procedures to which the tissue is submitted during its preparation for electron microscopic investigation. This may well explain the differences in the findings of different investigators. The figures represent fairly well however the average magnitude of the measured values and in this regard the results of different authors are yielding corresponding results very well.

## VI

### ULTRAMICROSCOPIC TRANSVERSE STRIATIONS OF MUSCLE FIBERS

In cardiac muscle as in skeletal muscle the familiar striations of the cross striated muscle fiber are determined by the cross striations of the myofibrils. Two types of cross striation must be differentiated—those of ultramicroscopic and those of microscopic order of magnitude.

In the ultramicroscopic order of magnitude we have the transverse rhythm of the protofibrils first described by Hall Jänius and Schmitt (1946) as spaced approximately 400 Å apart in the fibrils of desiccated skeletal muscle. According to investigations by Draper and Hodge (1949), this ultramicroscopic cross striation is a generalized occurrence seen in metal shaded preparations of desiccated skeletal muscle as well as in ultra thin sections of skeletal muscle. According to these authors the distance between the ultramicroscopic cross striations is dependent upon the state of contraction of the myofibril. The transverse periodicity of striated muscles seems to affect more than the protofibrils because according to Hodge it also involves the tiny bridges which bind the protofibrils together (1956). In the striated muscle of man (psoas and pectoralis) I have been able (1957g Figs 7 and 8) to demonstrate ultramicroscopic rhythmicity in bands separated by approximately 250–300 Å. In cardiac muscle I first observed these striations in the auricle of the bat (1954) where their spacing was approximately 250–300 Å. In the guinea pig I found the striations (1955a, b) in the auricular and ventricular musculature (300–350 Å) and I likewise found them in as yet unpublished studies on the auricle and ventricle of the mouse. Recently this rhythmicity of the heart muscle fibril (250–300 Å) has also been confirmed by Hodge (1956).

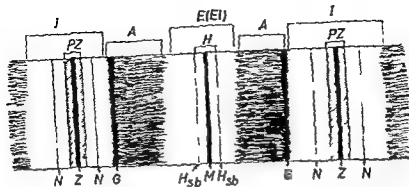


Figure 12 Diagram representing in schematic fashion the zones of a sarcomere. Most of these zones are visible in various portions of Figure 13

Z zone also appears as the darkest of the striations. It would seem that the muscle fibril is especially brittle at this point. In cases where some force is applied to the tissue under examination one most frequently sees tears or separations of the myofibril in the region of the Z-zone. In such an event as illustrated in Figures 14, 15 and 16 Z either remains attached to one piece of the broken fibril or else it becomes detached from both pieces thereby actually appearing as a disk (see Fig 16 at the arrow) however it does not appear as a disk crossing the muscle fiber but only the myofibril.

Approximately midway between two Z-bands one usually finds a second sharply delineated line which light microscopists have referred to as the M zone ( $M = \text{Mittelscheibe}$ ) even though M like the Z is neither a true band nor a membrane in the usual sense of the word. As a rule M is thinner than Z.

Two lighter zones approximately equal in width are found one on either side of the Z-zone. These light zones have been given the name I ("Isotropic Zone"). Within the I zone one finds on either side of Z one rarely two thin lines designated as N by their discoverer Engelmann ( $N = \text{Nebenscheibe}$ ). Frequently one finds a thin dark zone but lighter than Z directly adjacent to Z and occasionally extending to the immediate border of N. This zone was designated as PZ (Para Z-band zone) by Hirsch and Philpott (1954).

On either side of M one finds a light zone H named after its

## VII

### THE MICROSCOPIC CROSS STRIATION

The literature concerning the discovery, via the light microscope, of the striation of skeletal muscle is very extensive (see the summations by Cohnheim, 1865, Kruse 1868 G Retzius 1881 Melland 1885, Rollet, 1885 Koelicker, 1888 McDougall 1897, Meigs 1908, Heidenhain 1911, Huggquist, 1931 Bärer 1948 Jordan 1920 1934, 1955 and many others). The nomenclature has not at all times been equally standardized. The diagram in Figure 12 is meant to show the manner in which we apply the usual nomenclature to cardiac muscle. As regards the myofibrils it should be stated at the outset that we have thus far been unable to find significant differences microscopically or ultramicroscopically between those of cardiac and those of skeletal muscle as to their longitudinal and transverse striations with the exception of the Z bands which will be discussed below. This fact was already apparent in the studies made upon dissociated cardiac muscle (Berms Evans Janney and Baker 1949).

The repetitive order of the various striations produces a segmentation of the muscle fibril, and therefore also of the muscular fiber dividing all into a succession of identical minute units the so called sarcomeres. In the older literature the sarcomeres were also referred to as little muscle boxes (*Muskelkastchen*). The boundary line of each sarcomere is the densest of the dark cross striations. This line has been well known since the time of Dobie (1849) and Kruse (1868) and designated as the Z zone (the Z strands for Kruse's term *Zwischenscheibe*). The word *Scheibe* means disk. However none of the zones of the striated muscle or of the heart muscle is really a disk crossing the muscle fiber.

In the electron microscopic picture (with osmium stain) the

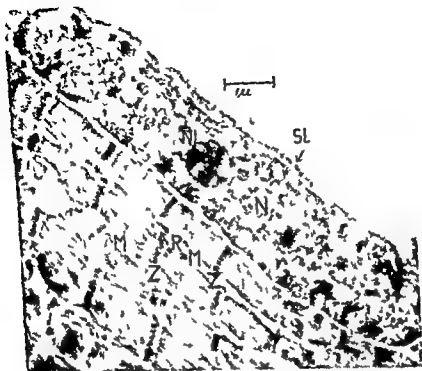


Figure 14 Muscle fibrils from the human pectoralis muscle Directly beneath the sarcolem (SL) there is a nucleus (N) containing a nuclear body (nucleolus NL) The myofibrils clearly demonstrate the Z and M bands and also the longitudinal striations determined by the protofibrils R = endoplasmic reticulum 13 000x

discoverer Hensen Within this light zone and parallel to M on either side of the dark zone one can recognize one occasionally even two stripes These are designated as Hsb (H sub-bands) by Hall Jakus and Schmitt (1946) and Draper and Hodge (1949) These same stripes were designated by Beams and his co workers (1949) as X stripes Between H and I one finds a broad dark zone Zone A (anisotropic zone) which at its boundary with I shows occasionally a distinct darker stripe which I have designated as G (*Grenzstreifen*) (see Figs 12 17 18)

This maze of zones at first somewhat confusing but regular repetitive and probably characteristic occurring within the



Figure 13 Myofibrils from the ventricle of a guinea pig. The individual zones can be clearly recognized and are labeled with appropriate letters. The dark stripes surrounding the Z zone represent the par Z band zone (PZ). M = middle line SS = sarcosomes 32 000 $\times$



Figure 16 Fibrils of human pectoralis muscle. At points indicated by arrows the Z zone is separated from both adjacent sarcomeres. At the upper end of the picture the fibers have been torn in very close proximity to the missing Z zone. M = middle line. 29 000×



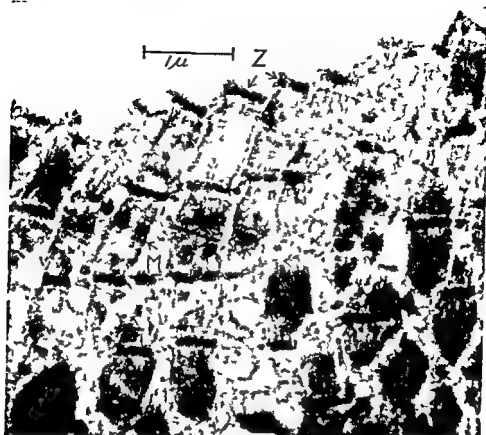


Figure 15 Fibrils of human pectoralis muscle torn directly at the Z zone  
20 000X

sarcomeres are found in cardiac muscle both of the auricle and of the ventricle (Bruno Hirsch since 1951 Lindner 1957 Moore and Ruska 1957) and also in skeletal muscle. The biologic significance of these zones is not yet known but hints at rhythmic biochemical differences within the sarcomere.

Figure 12 summarizes all of the above in a simplified fashion. As far back as 1933 Jordan observed a widening of the H zone upon stretching of skeletal muscle. This was confirmed electron microscopically by Perse and Baker (1949). D. Philpott and A. Szent Gyorgyi (1953) linked this zone which upon expansion of the muscle is clearly visible between H and A with the elastic elements of the muscle fibril and called it Zone E (A. Szent

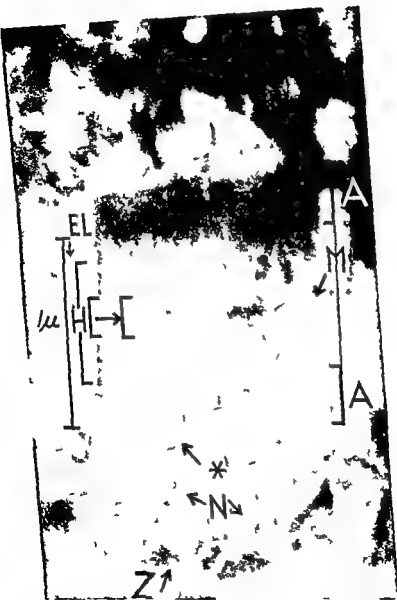


Figure 16. Myofibrils of the same psora muscle (newborn) illustrating a different state of muscular tension. Between the two A zones there is at either end a broad light zone (EL). Again here the dark G band (†) is visible running parallel to the N band and bounding the A zone. 50 000×



Figure 17 Muscle fibrils of human psoas muscle showing all the typical zones. One can clearly recognize Zone N running parallel to the Z band and a dark stripe marked with a star where Zone A bounds on Zone I. This boundary line has been designated as G (Grenzstreifen). In the region of the Z band Z band sarcosomes are visible singly or in pairs (SS).

32 000 $\times$

(B. Hirsch 1951) and can be best demonstrated in ultramicroscopic teasing preparation. Pease and Baker (1949) also claim the existence of such Z bands in skeletal muscle. It remains to be proven however that these Z bands of skeletal muscle are as constant as are those of cardiac muscle. Although the insect's flight muscles show the Z zone very clearly as in Figures 21, 22, 27, 27a, our own research has failed to discover distinct Z bands connecting the individual myofibrils in the flight muscles of the house fly, the bee, the bumble bee, the wasp and the hornet (Hirsch 1953c). Chapman's (1954) photographs of the flight muscles of various insects agree with these observations. It seems that the same is true for the myofibrils of the Purkinje Fibers (see below) in the mammalian heart.

The Z bands and fibrils of cardiac muscle produce a wave pattern in which the Z bands are connected on either side with the sarcolemma. I have therefore spoken of the structure of the heart muscle (myocardium) fiber as being similar to a warp and wool pattern. That these Z bands cannot be demonstrated in every longitudinal section of cardiac muscle is secondary in importance to the fact that they are frequently very noticeably demonstrable (see Fig. 4). This is equally true of the human heart as it is of the hearts of all vertebrates investigated thus far. I have been emphasizing this point repeatedly since 1950 and recently it has been confirmed in the rat heart by Hodge, Huxley and Spiro (1954). There are several reasons why the Z bands are frequently not visible. The most important reason seems to be that these bands are in reality bands and not plates. Therefore with our ultra thin sections we do not always manage to catch the bands since they are neither always found lying in the direction of sectioning nor apparently are all the bands always found to be running parallel in the same direction. The connection of the Z-bands to the sarcolemma has been found again and again (Hirsch 1951 & in Breemen 1953; Hodge, Huxley, Spiro 1954). One result of this attachment is that the sarcolemma of cardiac muscle fibers when sectioned longitudinally often appears as a serrated edge (garland shape). The indentations of the garland are as one can clearly see from our illustrations attached to the Z-band. The physiological result of such an

Gyorgy, 1953) These same authors also connected the changing thickness of Zone I with the elastic elements of the muscle fiber and with their tone (1953) Our Figures 17 and 18 show varying degrees of stretching of the fibrils of the same human psoas muscle

Unfortunately Rollet, in 1885, already used the designation E to signify a different striation found in the neighborhood of the Z-band It would perhaps therefore, be better to designate the new zone, linked with the elastic elements of the muscle as E1, rather than as E

Of all the above mentioned zones and bands only the Z stripe, the boundary of the sarcomere, can be relied upon as a constant and unchanging phenomenon In good photographs, M also is usually though by no means always present Whether it is justified in the absence of all other distinguishable zones to designate a uniformly dark region stretching from one Z to another as A, is I think, open to much questioning In summary it can be said that Z is always present in electron microscopic pictures of the muscle fibril (skeletal and cardiac muscle), while the ability to demonstrate all the other zones of the sarcomere is dependent upon the condition of the muscle at the moment of fixation Tension or relaxation activity or inactivity of the tissue being examined, all strongly influence the possibility to demonstrate any of the zones except Z In the course of embryological development it would appear that Z is the first demonstrable stripe (Van Breemen 1952) As far as we know, Z is present not only in the muscles of vertebrates and insects but it is also invariably present in the muscles of the crustacea (Farrant and Mercer 1952) The physiological significance of the variability of the striations in striated muscle as regards the function of the striations has been the subject of many studies and much speculation for over a century although as yet the problem has not been satisfactorily solved

As previously mentioned the ultramicroscopic structure of the cardiac muscle fibril is essentially identical with that of the skeletal muscle fibril of the same animal This is equally true of the auricle and of the ventricle Cardiac muscle however has a constant finding of thin bands in the region of the Z zone which connect the myofibrils of the myocardium with one another

arrangement may well be that for example in the heart where due to the sarcosomes the myofibrils are but loosely picked together the structural order may still be maintained during contraction much as Perse and Biker (1949) suspected to be the case for skeletal muscle. I have emphasized this for cardiac muscle repeatedly (1952a b 1952c). Szent Gyorgyi has also recently championed this idea (1956).

The fact that the Z zone the Z band and when present the PZ zone are always found approximately in the center of the light I zone leads one to speculate that the I band—and also the Z zone—may have something to do with the origin of the I zone (Kisch 1951 1957g). It must be said however that nothing certain is known about this. It would also be pure conjecture to argue that the relation of the Z zone to the N zones or that of the M zone to the Hb zones reminds one of the formation of the layers which bear the name of Liesegang (*Liesegang'sche Schichtung*).

Commonly in pictures of insect wings and more rarely also in pictures of cardiac muscle one can see that the protofibrils appear to pass through both the Z and the M zone and not just through the latter. In this region the protofibrils are thickened and the substance between them is darker than is the case outside of Zone Z (Kisch and Philpott 1955 Lindner 1957). It is not yet possible to decide whether we are dealing here with concentrations and encrustations of minerals as Hodge (1949) has postulated.

The great variation in appearance of myofibrils even within the same muscle is illustrated by our photographs of various myofibrils of the same human psoas muscle. According to Philpott and Szent Gyorgyi (1953) the variable expansion of the E1 zone is proof of the fact that different myofibrils of the same section of muscle were in different degrees of tension at the time of fixation (see Figs 17 and 18).

It is well known that Zone I and Zone II can both disappear completely when the muscle is in its contracted state. Sometimes M also disappears and then only the bounding Z zones remain. Zone II will occasionally appear with sharp and clear borders while M is missing (see Fig. 19 from the heart of a pipe fish).



Figure 19 - Ventricle of a pipfish. Myofibrils and sarcosomes. The M line is missing from the clearly recognizable H zone. 25 000 $\times$

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## VIII

### PSEUDO STRIATION OF THE MYOFIBRILS

A J Hodge and his co workers (Hodge, Huxley and Spiro 1954, Hodge, 1955) were perhaps the first to point out the fact that in addition to the well known cross striations of muscle fibers electron microscopic pictures may contain striations which are artifacts and have nothing to do with the organic structure of the myofibril. They designated this form of striation as pseudo striation.

Hodge (1954) explains these pseudo striations by postulating that a muscle fibril has been cut at an angle by the ultra thin sectioning technique whereby its regularly ordered protofibrils have also been cut at an angle row upon row. Thus the section cuts the protofibrils arranged in rows in a step wise fashion which produces a picture of a succession of light and dark bands. Our schematic drawing (Fig 20) attempts to show this. If the pseudo striations take the form of concentric arcs these same authors (1954) claim that a twisting of the material in the course of fixation and embedding is to blame.

Our Figures 21 and 22 show cases of these pseudo striations. I do not believe that they could result from either an ultra microscopic crumbling of the specimen or from regular variation in the thickness of the section attributable to vibration of either the microtome or the specimen in the course of sectioning. In the former case the periodicity of light and dark seen occasionally would have to affect the whole section sarcomeres and muscle fibrils alike. This however is not so. In the latter case it would be hard to understand how the pseudo striations could achieve an order of concentric arcs.

I believe that the pseudo striations could be the result of

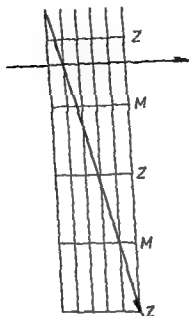


Figure 20 Diagrammatic representation of a cross section and diagonal section through the protofibril of a muscle fiber

several widely different phenomena of which only two will be discussed here

First a parallel arrangement of bands can doubtless result as Hodge has emphasized (1954 1955) from oblique sectioning through muscle fibrils wherein the section cuts across adjacent rows of protofibrils in step wise fashion and the interspace between the rows appears electron optically less dense than do the protofibrils themselves This is illustrated in our Figures 21 and 22

Second in isolated cases we may be dealing with a rhythmic succession of zones more or less densely stained by the osmium or other stain In this connection we should think primarily of the cases showing wide concentric arcs Such an appearance would be particularly likely in sections where the dark pseudo striations make recognition of the cross section protofibrils impossible provided that the penetrating osmium solution is producing the phenomenon of Liesegang layers in these sections



Figure 21 Flight muscle of a meat fly. Note the pseudo striations. These are explained in part by the diagonal plane of sectioning, in part by a distortion of the fibrils through the plastic embedding material and in part there is no explanation for them. Note also the cytoplasmic matrix in which the sarcosomes (SS) are embedded. 13,000 $\times$

Several things are in favor of such an interpretation. For one it allows for the fact that the striations may occasionally run almost parallel to the longitudinal axis of the fibril. More important however is the fact that one occasionally sees as in our Figure 21 that the concentric arcs become more and more widely separated from one another the further out they are from the imaginary center. This exactly is a typical property of the Liesegang layers when they occur by precipitation of a suitable

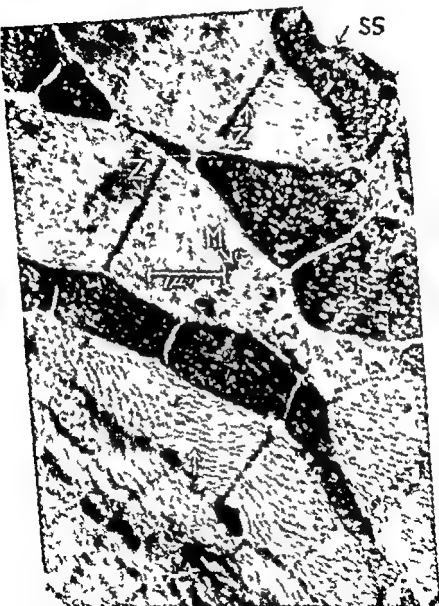


Figure 22 Flight muscle of a meat fly. Sarcosomes are visible between the myofibrils. Note the plastic deformity of the sarcosomes and two intersecting groups of pseudo-striations indicated by an arrow. 20 000 $\times$



Figure 23 Myofibrils of the human uricle. Some of the Z bands are clearly connected with the sarcolem 32 000X

substance infiltrating into a colloid medium. A similar effect could be achieved however if an obliquely sectioned myofibril were bent within the embedding material. The internal tension thus built up could lead to a fan like spreading of the protofibrils within the myofibril. Ordinarily, with the usual undisturbed arrangement of the myofibrils the protofibrils would have run parallel to one another. The result in both these instances would be the same. In the postulated oblique section the formations of protofibrils would be increasingly separated from one another as the distance from the center of tension increased.

One should also note the not easily understandable crisscrossing of various pseudo striations within the same sarcomere as illustrated in our Figure 22 (arrow.)

Finally we should also point out that the pictures of pseudo-striations both Hodge's and Chapmann's (1934) and ours are all seen in the flight muscles of insects. I have never observed the above described concentric arch formation of pseudo-striations as clearly in any other muscles. On the other hand I long ago observed and reported on the parallel striations which may result from the transverse sectioning of a myofibril of the heart (1934a)

## IX

### THE SARCOSOMES AND THE SARCOSOME THEORY OF THE FUNCTION OF THE HEART

Since the significance of sarcosomes to date still remains largely unrecognized among physiologists, pathologists, and cardiologists despite the fact that they have been proved to be vital components of the heart muscle fiber (B. Kusch 1951, 1952, 1957g) it is necessary here to discuss these structures in some detail.

#### A NOMENCLATURE

The story of the discovery and investigation of sarcosomes, especially the much studied sarcosomes of the flight muscles of insects, will not be gone into here, as we thoroughly covered it in 1952 (1). In order to avoid misunderstandings, however, the nomenclature requires clarification. Sarcosomes were so named in the year 1890 by the outstanding Swedish anatomist Gustav Retzius. They are the tiny, so-called interstitial granules of the protoplasm, the largest of which are visible even through the light microscope. They have been known to light microscopists since the year 1840 (Bowman 1840, Henle 1841, Koelicker 1851, and many others). As these granules are found in the protoplasm of muscles, G. Retzius called them sarcoplasmisomes, and abbreviated this name to the linguistically hindered word *sarcosome*. For historical reasons, and also out of respect for an outstanding scholar, we are strongly inclined to continue the usage of this name, and indeed there is no good reason for replacing this term by one which would probably be less correct.

The term mitochondria, frequently used to designate the tiny

inclusion bodies in the protoplasm can be literally translated as ribbon shaped structures" Especially in insect muscles how ever most of the sarcosomes are anything but ribbon shaped Since the sarcosomes of insect muscle are exceptionally large it is very simple to convince oneself of this fact with a light microscope

I was able to show the high degree of plasticity of the material from which the sarcosomes are built (1957g). Therefore sarcosomes that are spherical in free suspension may be squeezed into any shape by the pressure of neighboring organelles (see Fig 22). The same is true for the majority of sarcosomes of cardiac muscle. Philologically speaking therefore it is not justified to designate a spherical or an egg shaped object as a ribbon.

These then are the reasons why the sarcosomes of cardiac muscle must not be designated as mitochondria though occasional ones might be seen to possess a mitochondrial shape. Therefore as has been our custom the little bodies composed of a sub structure which will be discussed in the following chapter will be designated as sarcosomes whenever they occur in muscle fibers. Where the little bodies are found in the protoplasm of non muscular structures (for example in the wall of the capillary) they will be designated as plasmasomes or if they really show a ribbon shaped appearance even as mitochondria.

## B LOCATION STRUCTURE AND FUNCTION OF THE SARCOSOME

The extensive literature concerning the light microscopy of sarcosome (summarized by Kisch 1952a) was until the end of the Nineteenth Century concerned mainly with proving the existence of these bodies. Even this did not go altogether unchallenged although the majority of histologists became convinced of the existence of sarcosomes at the end of the Nineteenth Century thanks mainly to the outstanding investigations of G hetzius (1890) Holmgren (1907-1910) Meigs (1908) and others. The exact location of the sarcosomes within the muscle their structure and their function however remained in doubt or totally unelucidated.



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#### A NOMENCLATURE

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The term *mitochondrion*, frequently used to designate the tiny



Figure 25 Left ventricle of an albino mouse (Ultramicroscopic teasing preparation) This poorly fixed preparation illustrates myofibrils and the sarcosomes which lie in between them. The well preserved Z bands (Z) aligned with the myofibrils in wavy and wavy pattern are especially noteworthy. TR = ring shaped structure. 13,000X

tween individual myofibrils or between myofibrils and the sarcolem and can be aggregated in large quantities in the pockets of the sarcolem as we for example have found to be the case commonly in auricular muscle and in the striated muscle of the diaphragm (1955a, 1958a, f). Figures 24, 25, 26 illustrate an aggregation of sarcosomes in cardiac muscle.

In muscle fibers especially rich in sarcosomes such as those of



Figure 24 Human auricle The sarcolemma (SL) presenting a double line is seen in several places to be connected with the Z band of the neighboring myofibril Sarcosomes are seen to lie between the myofibrils 18 000x

### *Where are the Sarcosomes in the Heart?*

After years of personal investigations on this subject we can state that the sarcosomes are always found within the muscle fiber and never within the myofibril (B Kisch 1951) or between different muscle fibers except for artifacts They are found be



Figure 26 Ventricle of the guinea pig. The mass of sarcosomes is in this picture much bigger than that of the scanty myofibrils. 22,000X

the heart or the diaphragm the sarcosomes are often found lying in long rows between the myofibrils. There they are tightly packed together and since their substance is evidently very plastic in nature, they often appear in the electron microscopic picture, to be impinging upon and flattening one another when ever a large number of sarcosomes lie close together. Our Figures 22 and 34 illustrate this clearly in the vertebrate and also in insect muscle. Another mechanical result of the existence of particularly large numbers of sarcosomes is that the individual muscle fibrils are forced apart by these small interposed bodies. A comparison of our Figures 32, 36 and 39 and in contrast Figures 33, 34, 41 and 42 show that in muscles which are poor in sarcosomes (such as the skeletal muscle of the mouse or of the guinea pig or of the rat or of man) the myofibrils of the muscle fiber are packed closely together whereas in the cardiac muscle of the same animal this is not the case as the sarcosomes interpose themselves between the myofibrils. These facts emphasized by this author since 1951 are recently corroborated by pictures of Moore and Ruska (1957) of Porter and Palade (1957) and others.

In sarcosome poor muscles the sparse sarcosomes are found mainly in the region of the Z zone occurring singly or in pairs (B. Hirsch 1955c, 1956f, 1957g, more recently G. A. Edwards and co-workers 1956, Porter and Palade 1957). Often where the Z bands are visible the sarcosomes appear to be attached to these bands. I have therefore called these the Z form of the sarcosome or Z sarcosome for short. This is illustrated for human skeletal muscle in our Figures 17 and 18 for mouse skeletal muscle in Figure 28 for guinea pig skeletal muscle in Figure 32 and for rat skeletal muscle in Figure 35. These Z sarcosomes are as a rule, much smaller than are the majority of sarcosomes found in the muscle fibers of the heart chambers although in the auricular muscle the sarcosomes are of a rather polymorphic appearance. We will have more to say about this later. In addition to the smaller ovoid Z sarcosomes skeletal muscle also possesses the band shaped type of sarcosomes (mitochondrial type).

In the heart as in other muscles great quantities of sarcosomes are always in the closest neighborhood of each nucleus (Hirsch 1951). This seems to apply to nuclei in general for in capillary

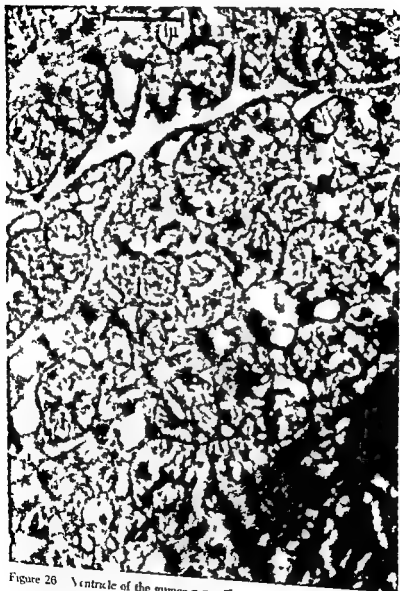


Figure 26 Ventricle of the guinea pig The mass of sarcosomes is in this picture much bigger than that of the scanty myofibrils 22 000×

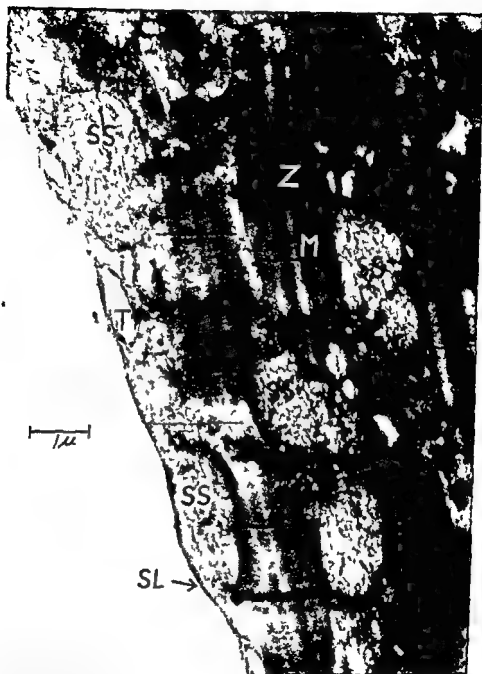


Figure 27 Flight muscle of a hornet. Note the large sarcosomes lying between the myofibrils, and the absence of Z bands in the presence of distinct Z M and H zones (H zone is the light zone on either side of the M)

SL = sarcolem T = Capillary of the tracheal system 13 000×



Figure 27a Flight muscle Honey bee Like in the heart muscle of the investigated vertebrates rows of sarcosomes fill the spaces between the myofibrils T = Capillaries of the tracheal system 14 000X



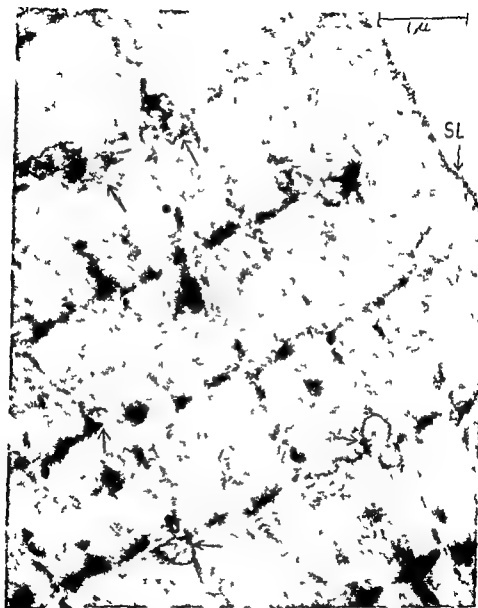


Figure 28 Leg muscle of an albino mouse. The sparse sarcosomes occurring in the region of the Z zones of the myofibrils are indicated by arrows.  
SL = Sarcolem 22 000x

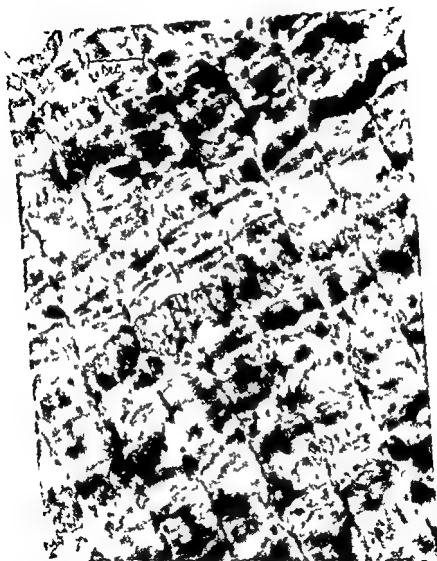


Figure 29 Diaphragmatic muscle of the same animal as in Figure 28. The sarcosomes are much more numerous, being partially small in the region of the Z zones and partially large, lined up in rows. Sarcosomes are seen in both the round and ribbon shape. The ribbon shaped sarcosomes are of the mitochondrial type. 15,000x.

endothelium one also regularly finds plasmosomes in the region of the nuclei which would seem to point to especially high metabolic changes within the nuclei or wherever they are found (Kisch 1951 and later) (see Figs 30 and 36)

The great quantity of sarcosomes in cardiac muscle is, presumably also responsible for the fact that this muscle is much harder and firmer than any other muscle in the body. The great firmness of cardiac muscle was already observed by Galen who thus explained the great working ability of the heart. If this peculiar consistency of the heart is as I presume the result of its enormous content of sarcosomes then Galen without ever understanding this connection was in the end quite right in his assumption.

### *Form and inner Structure of the Sarcosomes*

Thanks to electron microscopy we now recognize the fact that sarcosomes and plasmosomes including the so called mitochondria are not merely homogenous droplets or rods of indeterminate substance of microscopic or sub microscopic size. It was formerly thought that the substance of which sarcosomes are made was most probably fat, glycogen or albumin. The electron microscope has shown that the sarcosomes are structures with a characteristic external form and internal structure (Sjostrand 1953 Palade 1952b Rhodin 1954 Kisch and Philpott 1955 etc.)

The recognition of the above facts enables us today to distinguish between sarcosomes and other microscopic and sub microscopic cell inclusions. Of course the recognition of a certain inner structure is not the only method for differentiating and recognizing the sarcosomes as such in each particular case. One can also draw many conclusions from the form and the location of these bodies alone. Indeed it is often difficult to recognize the typical internal structure of the sarcosomes; this requires both an excellently prepared specimen and excellent photography. The inner structure of the sarcosomes changes apparently with the changes of their function too.

Concerning the form of the sarcosomes we must first emphasize the fact that the method of ultra thin sectioning employed in electron microscopy never allows one to see an entire sarcosome



Figure 30 Ventricle of the same mouse as shown in Figures 28 and 29. The spaces between the myofibrils are filled with sarcosomes. In the region of the nucleus (V) the sarcosomes are especially numerous. 13,000x.



Figure 31 Auricle of the same mouse shown in Figures 28, 29 and 30. Note the large number of sarcosomes between the myofibrils and also between the myofibrils and the sarcolemma (SL). In the upper right hand portion of the picture there is an interstitial cell (I) containing a nucleus (N) surrounded by many plasmasomes (PS). This cell sends out its extensions to the interspace between the muscle fibers. C = Capillary. M = Myofibrils.

7,000x



Figure 32. Leg muscle of a guinea pig. It is not possible to identify a single sarcomere between the myofibrils with any degree of certainty. 20,000X.



Figure 33 Eye muscle from the same guinea pig shown in Figure 32. Numerous sarcosomes, mostly of the mitochondrial type, are found between the sarcolem (SL) and the myofibrils and between the individual myofibrils. 20 000x



Figure 34 Ventricle of the same guinea pig shown in Figures 32 and 33. Masses of sarcosomes are seen lying between the myofibrils. Note the double-ring form and canal form of structures between the sarcosome indicated by arrows. 25,000X.



Rather, what one sees in the electron microscopic image are mere slices, cut out of the sarcosome, much as slices of bread are cut from a whole loaf. Just as one could say very little about the form and size of a loaf of bread or of a sausage from seeing only a slice of it, it is very difficult to say anything about the form and size of an entire sarcosome from the picture of a single slice of the sarcosome obtained in sectioning for electron microscopy. The form and size of the slices we view are always dependent upon the plane in which the sectioning knife chanced to strike the sarcosome. The comparison of a series of many consecutive electron microscopic pictures of the same tissue however permits one to draw certain conclusions.

For example, if the sarcosomes of the heart chambers in the vast majority are seen to be round or oval, irrespective of whether the myofibrils between which they are found are sectioned longitudinally or transversely then we are justified in concluding that the sarcosomes of the heart chambers or at least the majority of them are spherical or ellipsoid in form. If, as is occasionally the case in the muscle, the picture shows a group of very small sarcosomes lying packed close together then we can be sure that we are dealing with small sarcosomes and not with tangential sections of larger sarcosomes as the latter would be a geometric impossibility in the case of a cluster of such small bodies. If, as our pictures show, tightly packed sarcosomes are flattened at their adjacent edges (see Fig. 41) or as in insect muscles, they assume bizarre forms which exactly fill out the spaces between muscle fibrils (see Fig. 22) then we are justified in concluding that the material of which sarcosomes are made up is of a plastic nature offering little resistance to a molding force.

It should also be emphasized that there is a difference in the form exhibited by the sarcosomes not only in different organs of the same animal but also in the same organ of different animals and that these differences are of a characteristic nature. Thus as we have just mentioned the sarcosomes in the ventricle of various mammals which we have investigated including man are predominantly round or ellipsoid while, as I was able to show with Philpott in 1955, the ventricle of the frog has sarcosomes



Figure 35 Leg muscle of a rat. At only two places within the cytoplasm between the myofibrils is it possible to see any sarcosomes ( $\times 27\,000$ ).



Figure 36 Diaphragm of the rat. Many sarcosomes between the myofibrils. Arrows indicate those of mitochondrial type. Notice the group of sarcosomes at the left upper corner ( ) near to the nucleus (N). 15 000×



Figure 37 Heart muscle (ventricle) of the same rat as Fig. 36 Rows of sarcosomes between the myofibrils Arrows indicate endoplasmic reticulum 15 000 $\times$

which are predominantly ribbon shaped. Recently Szent-Gyorgyi has confirmed the same finding (1956)

This difference in the shape of sarcosomes of various tissues applies to the heart itself when we compare the auricles and ventricles of the same heart and may correspond to a different function of differently shaped sarcosomes.

In the ventricular musculature for example only a small portion of the sarcosomes assumes the elongated sausage shape (myochondrial) type while in the auricles many of the sarco-



Figure 38 Ventricle of the cow : Masses of sarcosomes near a nucleus (N) between myofibrils : Notice their inner structure 12 000X

somes are of this type. The elongated sarcosomes can be as long as  $3\ \mu$ . They are generally only  $0.2$  to  $0.3\ \mu$  in diameter. The round or ellipsoid sarcosomes of cardiac muscle have a diameter of  $0.5$  to  $1.0\ \mu$ , while the sarcosomes of the insect flight muscle have a diameter of about  $3$  to  $4\ \mu$ , a fact which explains why histologists have in the past always favored these particular sarcosomes in their investigations. They are a challenge to the electron microscopist too (B. Kisch, 1959). It is quite typical of the auricles that their muscle fibers exhibit a great variation in the form and size of the sarcosomes (Polymorphism of the Sarcosomes B. Kisch, 1955, 1956a, b, 1957g) recently corroborated for the atrium of the turtle by pictures taken by Fawcett and Selby



Figure 38a Human ventricle Sarcomeres between myofibrils arrows indicate profiles of endoplasmic reticulum 23,000x

(1958) We will later make reference to the fact that differences between the sarcomeres in the auricle and ventricle of the same heart support the earlier contention of this author that the biochemistry of the auricle and of the ventricle of the same heart are not necessarily identical (1956a)

The inner structure of the sarcomere has been variously investigated as has the structure of the so called mitochondria of the non muscular tissues such as liver kidney and glands (Sjostrand



Figure 39 Sarcosomes from the heart of a cow. Notice their outer membrane and inner lamellar structure. 30 000 $\times$

1953 Palade 1952b Rhodin 1954 Napolitano and Fawcett 1958 Kisch 1959 and others)

Palade named the lamellae which were most easily recognizable in sections of sarcosomes. Cristae mitochondriales. It is the hallmark of sarcosomes that they are made up of a membrane surrounding an inner content. This fact had already been recognized by light microscopists investigating suitable sarcosomes (Koelicker 1886 see Kisch bibliography 1952a). In the sarcosomes of insect muscles however it is often impossible to demonstrate a distinct membrane. This seems not to be an optical artifact only (Kisch 1959). In the mammal when the content of the sarcosome is removed by osmosis one can see the membrane which remains looking for all the world like an empty sac. This same phenomenon is the case within a very short time after the death of these animals and this author has called these sac-like empty membranes ghosts of sarcosomes (1955c). The internal lamellae of the sarcosomes which are markedly osmiophilic are



Figure 46 Sarcosomes from the heart of a guinea pig. Note the tear in the external membrane indicated by an arrow and the concentrically arranged perpendicular cut lamellae of which each can be clearly recognized as having two surface boundaries. Their width is approximately 150 Å. Their dark surface boundary is approximately 50 Å thick. The two sarcosomes visible in this picture are embedded between obliquely sectioned myofibrils of the heart whose parallel protofibrils and Z bands are easily recognized. 58,000 $\times$ .



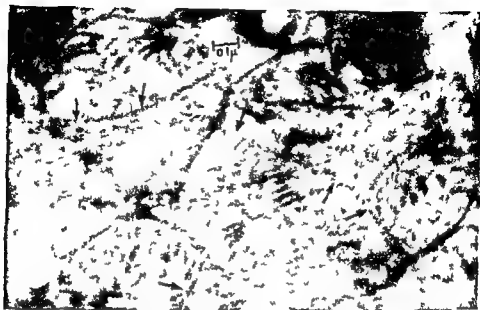


Figure 41 Sarcosomes of the guinea pig heart. The arrows point to lines which might be regarded as the boundary lines of dividing sarcosomes  
55 000 $\times$

closely packed together in the sarcosomes of the heart and of the flight muscle of insects. On cross section they have the appearance of a double line, each line having a thickness of approximately 50 to 75 Å. The internal lamellae of the sarcosome surround as they lie by a much less osmiophilic matrix, are sometimes seen to extend diagonally or transversally across the entire sarcosome. At other times they are seen to be connected with the sarcosome membrane on one side only, and sometimes they don't seem to touch the membrane anywhere. At times, they are arranged in parallel lines, while sometimes they are arranged in concentric circles (Kisch, 1955, etc.) or in radiating rays. Because of this I prefer to use the term *internal lamellae* rather than *Cristae*. I have often pointed out the similarity between these pictures and the trichites of Otto Lehmann (1954a, 1954b and elsewhere). The content of the sarcosomes is occasionally strongly reminiscent of Lehmann's liquid crystals. Occasionally, however, one finds no lamellae at all within the sarcosome. Rather, one finds rod-shaped pieces of lamellae or fragmented particles within an osmiophobic homogenous matrix. This may



Figure 42 Sarcosomes between myonoblasts of a meat fly. Notice the arrangement of the lamellae in the sarcosomes. T indicates cuticular system of the tracheal system. 20,000 $\times$

be the case even when neighboring sarcosomes in the same picture exhibit well developed lamellae (see Fig 39). This observation makes one wonder whether the absence of a lamellar structure is in fact only an artifact and also whether really each well preserved sarcosome should exhibit a regularly ordered lamellar internal structure. It would seem much more probable that the variation in internal content is indicative of a variation in functional state of the sarcosome being viewed (Kisch 1954a, 1955a, c, 1956, 1957g, lately also Powers and co workers 1958).



Figure 43 Sarcosomes between myofibrils of a meat fly 10 000x

or even of degenerative changes of the content of the sarcosomes which may arise under pathologic conditions

Recently the fact that neighboring plasmosomes differ, one showing the inner lamellar structure, while the next shows only an amorphous content has been confirmed also by Napolitano and Fawcett (1958) for brown adipose tissue in the new born mouse and rat. These authors as well as Kisch (1959), have shown that even one individual sarcosome may in one place contain lamellae whilst in another place it contains only amorphous material. All such observations seem to confirm this author's previous opinion that the observed difference in content may be due to different functional states of the sarcosomes being viewed.

It should be emphasized however that necrobiosis tardy fixation after death and improper handling of the material all open the door to artifacts of swelling and disintegration which may go so far as to produce ghosts of sarcosomes. I cannot go along with the concept that ultramicroscopic canals exist in the plasmosomes in which metabolic exchanges and metabolic processes occur which in turn regulate the muscle metabolism.

### *The Function of the Sarcosomes and a Correlation Between Sarcosomes and Muscle Function*

Even in the earliest electron microscopic investigations of sections of cardiac muscle (Kisch 1951 1952) the most striking



Figure 44 The internal structure of the sarcosomes of a wasp caught in the summer time. The sarcosomes have a striking corrugated and wavy internal lamellar pattern. The letter T denotes a branch of a capillary of the air transporting tracheal system. (From Kisch and Philpott 1955) 34 000×

feature was the enormous quantity of sarcosomes within the muscle fibers of the heart. After examining countless pictures I would today somewhat allegorically state the case as follows: whereas in the electron microscopic picture of cardiac muscle one often has to search amongst the sarcosomes for the individual muscle fibrils, in the picture of skeletal muscle one must search



Figure 45 Sarcosomes from the human ventricle. Note the double limit of the inner lamellae. (From Kisch, Crivusoglu and Marangoni, 1959) 70 000 $\times$

among the myofibrils to find sarcosomes. A simple comparison of Figures 28 through 38, showing both cardiac and skeletal muscle of the same animal, will make this point quite clear.

All biochemical studies of cardiac muscle should also in future take cognizance of the fact that at least in certain places cardiac muscle contains a mass of sarcosomes which is equal to or outweighs the mass of myofibrils.

When working chemically on extracts of heart muscle one should be aware of the fact that *the material which is extracted is not only muscle fiber*. Rather in places the extract is made up

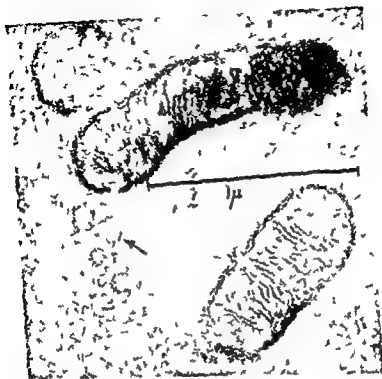


Figure 46 Plasmasomes from a thrombocyte of the mouse. The arrow points to cross sectioned channels in the cytoplasm. Notice the membrane of the plasmasomes and the double lining of the inner lamellae. 56 000X

of 50 or more sarcosomes or their extracts only the remainder being extracted of myofibrils. Extracts of heart muscle therefore do not permit of comparison with extracts of skeletal muscle for the purpose of deriving any conclusions about existing differences in the chemical contents of the myofibrils of these two types of muscle (Hisch 1956f 1957g)

In line with the above stated condition of the human heart muscle we should take into consideration one other fact. In nature a similar occurrence of a huge mass of sarcosomes in which myofibrils are embedded is found only in the flight muscle of insects. Linking these two facts together I came to the conclusion in 1951 that sarcosomes must be essential to the intensive and ceaseless activity of these two otherwise seemingly unrelated muscles. Originally one had suspected that the sarco

somes were either depots for energy rich material used by the strenuously and steadily exercising muscles, or else fatty degeneration products of the muscle fiber itself (see Kisch 1952a). In recent years however, various researchers have demonstrated for the mitochondria of other organs (liver, kidney), that their plasmasomes are in reality enzyme depots (literature, G. H. Bourne 1950). *This seemed to me to be the answer to one of the central problems of cardiology*. I assumed (1951) that the sarcosomes of the heart contained enzymes of the most vital importance to the heart's metabolism. The vast numbers of sarcosomes present in cardiac muscle compared to skeletal muscle together with their advantageous position within the muscle fibers would seem to make them ideally suited for effecting an immediate reversal of the chemical changes which occur in connection with each contraction of the cardiac muscle. This is more especially so as the usefulness of an enzyme within a cell undoubtedly depends upon its quantity and its localization within that cell. As we can see both the location and the quantity of sarcosomes within cardiac muscle are optimal. The mass of the sarcosomes exceeds the mass of the myofibrils at many places within the ventricle and all the sarcosomes are found to lie within the muscle fiber. It is because of this that under normal conditions one never finds bigger amounts of metabolites as chemical exchange products accumulating within the heart muscle. Therefore in contrast to normal skeletal muscle which possesses relatively few sarcosomes the normal heart can carry on strenuous work for a lifetime without requiring any lengthy pause to recover (Kisch since 1950).

Thanks to the outstanding work of biochemists the importance of this opinion has subsequently been thoroughly substantiated for the sarcosomes of both insect muscles (Watanabe and Williams, 1951, 1953) and cardiac muscle (Plaut and co workers 1952, Slater and Cleland 1952, Cleland and Slater 1953a, b, Siekevitz 1959). *Sarcosomes are carriers of metabolic enzymes and by means of their enormous quantity and favorable location within the heart they permit that organ to function without any lengthy rest period through an entire lifetime*. The reader is referred to the above mentioned references for biochemical details.

It is as yet too early to state definitely the mechanism by which the enzymes are released from the sarcosomes. It might be that part of the enzymes are located at the surface of the lamellae within the sarcosome and that they are released in response to biochemical and physicochemical changes in their environment. Or they might be located at the sarcosome membrane. In each case they may leave the sarcosomes by osmosis under favorable circumstances. It is also conceivable that both of these mechanisms may prevail to some extent. As previously mentioned the double interphase boundary of the lamellae which can be easily recognized in the pictures has a thickness of approximately 50 to 70 Å. This could correspond to the thickness of micromolecules of osmophilic enzyme proteins or enzyme lipoproteins which are located in these particular places. Whether the appearance of the internal structure of the sarcosomes changes with their enzymatic activity and whether perhaps these organelles might play a recognizable part in clinically proved insufficiency states of cardiac muscle is the present topic of our investigations (Kisch 1954, 1959). All of the above are new approaches towards the solution of important clinical problems in which experiment and electron microscopy together would seem to hold the ultimate answer. Upon the solution of these problems will depend the further development of many phases of cardiology. Of one thing we can be certain even at this point: within a very short time after death the sarcosomes lose their content and only the empty membranes remain giving rise to the so-called "ghosts of sarcosomes" (Kisch 1953).

A first attempt at solving this type of problem has led to the following histological results which are of basic importance.

Cardiac muscle and skeletal muscle are for all practical purposes two ends of a spectrum. Cardiac muscle works day and night without longer rest periods against the resistance of the blood pressure within the aorta and the pulmonary artery. In some small animals (such as the mouse, the rat, the hamster, the sparrow, the finch, and the sea gull) the heart beats at a rate of 300 to 1000 contractions per minute (Kisch 1953a). In contrast to cardiac muscle, skeletal muscle works under a requirement of substantial periods of rest. When such rest periods are not ex-



tensive enough skeletal muscle tires very easily. As has been pointed out, in line with this difference in function, cardiac muscle contains a very considerable quantity of sarcosomes while skeletal muscle contains very few. Among the striated muscles of the vertebrates, there are many which classified according to function would fall in between cardiac and skeletal muscle as described above. For example, the eye muscles function continuously while one is awake and the diaphragm functions continuously day and night. Naturally, the resistance against which the diaphragm must work is minimal when compared to the resistance offered the cardiac muscle. Electron microscopic studies have now revealed (Kisch 1956e f 1957g) that just as the function of the eye muscles and diaphragm falls in between the two extremes of cardiac and skeletal muscle function so also their content of sarcosomes falls in between the extremes. Their muscle fibers especially those of the diaphragm contain many more sarcosomes than those of skeletal muscle and yet their sarcosome content falls far short of that of cardiac muscle of the same animal. (See Figs 28 through 38.) The cardiac muscle of varying animals also exhibit typical variations in their sarcosome content. In the hearts of cold blooded animals (fish frog) I found far fewer sarcosomes than in the hearts of mammals (Kisch 1954), and in the ventricle of cattle (pulse rate 30-50) far fewer than in that of man or small mammals.

The statement about the astonishing abundance of sarcosomes in the diaphragm (Kisch 1956e f) has recently been confirmed by a picture of the muscle of the diaphragm of the rat published by M. L. Watson (1958).

All of these observations point out one basic fact *the ability of a striated muscle to do strenuous untiring work is in mathematical terms a function of the number of sarcosomes which it contains* (Kisch 1951 1954, 1956b f 1957g) and of the number of functioning capillaries per mm<sup>2</sup> of the muscle. The vital importance of the amount of sarcosomes in the muscle fiber is apparently still not well understood even by most of the scholars working on sarcosomes.

Finally, it should be mentioned that perhaps the varying forms of the sarcosomes which we observe may also signify varying



Figure 47 Section through a red blood cell of man. No plasmasomes and no endoplasmic reticulum noticeable. 36 000 $\times$ .

function. After all we are used to the idea that in nature generally difference in form signifies difference in function. It has already been mentioned that part of the sarcosomes of the auricle exhibit a different form and a different distribution than do the sarcosomes of the ventricle of the same heart. The typical sarcosomes of skeletal muscle the Z-sarcosomes are decidedly



Figure 48 2 Red Blood cells (R) in capillary of the human heart. No plasmasomes and no endoplasmic reticulum. In the capillary wall (C) a big vacuole, different plasmasomes and many small droplets. Arrows point to parts of endoplasmic reticulum and 2 slits in the wall (See p. 119 ff.)  
18 000 x



Figure 49 Capillary from the heart of an albino mouse. Within the cell wall the endothelial nucleus (EN), the mural slit (MS) and the plasmasomes indicated by arrows are visible. The capillary closely surrounds a white blood corpuscle whose nucleus and plasmasomes are easily recognized. 15 000x

smaller than are the sarcosomes of the ventricle and they are mostly located singly or in pairs at the Z zone.

We are still in need of a detailed study to allow inclusion within this picture of the most varied of animals and of the nonstriated muscles too. Our own as yet unpublished comparative studies point towards the fact that for example the internal structure of the sarcosomes of the flight muscles of insects may be dependent upon the season. This could be a factor in the decreased flying ability of certain insects in the fall of the year (e.g. flies).

Electron microscopic studies of the sarcosomes have led since 1951 to investigations which with surety have proven that these structures are a vital part of the cardiac muscle. It is however not this writer's opinion that the enzymes of the cells are located and built exclusively in plasmasomes (mitochondria).

In connection with the above, we should recall here that large numbers of plasmosomes are also found in the region of the nerve end plates of striated muscle as W. Kuhne has already shown decades ago. In line with current thinking about the method of transmission of nerve impulses I thought it likely (1952a) that the piling up of plasmosomes in this location was somehow connected with the production of the acetylcholinesterase which is required there. This close connection between plasmosome content and function of a cell can also be demonstrated in many other fields of physiology which can be mentioned here only in passing.

Of all the cells in the body the mature red blood corpuscles of mammals probably have the lowest oxidative chemical exchange and the lowest oxygen consumption. This is commonly connected with the fact that mature red blood corpuscles do not have a nucleus. This could well be a factor in the unique ability of the red blood corpuscles to transport oxygen. As far as we know, however, the red blood corpuscles of mammals also have no plasmosomes (mitochondria) while in contrast the white blood corpuscles of the same animals have nuclei and a vast quantity of plasmosomes. The lack of plasmosomes in mature red blood corpuscles of mammals must be another especially important factor in the low chemical exchange of these cells. The immature blood cells of the mammal (the reticulocytes) have a distinct oxygen consumption (Rimsey and Warren 1932) and they also possess plasmosomes of both a typical and an atypical variety (Chalfin 1956, Brunner and co-workers 1956, Brunstemer and co-workers 1956, this author's unpublished observations). One other thought comes to mind. O. Warburg demonstrated the abnormal oxygen consumption of tumor cells. A study of the plasmosomes of tumor cells could reveal as yet unknown morphological characteristics of these cells.

All the above raises the question of the relation between the nucleus and the plasmosomes of a cell. Since 1951 I have stressed the fact that numerous plasmosomes (sarcosomes) are always found in proximity to the nucleus. In mammalian red blood corpuscles both the nucleus and the plasmosomes are absent. Can not one of these two structures exist without the other? Can the



Figure 50 : A red blood corpuscle and a white blood corpuscle in a capillary of mouse lung. Within the white blood corpuscle are seen two sections of the nucleus (N) and numerous plasmasomes of which several are indicated by arrows. There are no plasmasomes seen in the red blood corpuscle.  
11 000×

plasmasomes exist only if a nucleus is present? This is doubtful according to the findings in blood platelets. Can the nucleus exist only if the plasmasomes are present? This is perhaps more probable. Perhaps though both the nucleus and the plasmasomes disappear simultaneously in the course of the development of the red blood corpuscle which also has no endoplasmic reticulum. It will require much work before one will be entitled to a definite opinion concerning the answer to these questions. In

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myofibrils or near the nuclei where ordinarily masses of normal sarcosomes can be found in the muscle fiber

*These findings not only prove the suspected role of the sarcosomes in the failing heart but give cardiology an entirely new concept concerning the mechanism of heart failure*

This author regards disintegrating sarcosomes also as a source of lipofuscin and some other lipids found by light microscopists in the heart of cardiacs and old people (See Chapter XIV)



this connection, it would be very useful to have histological studies made upon the nucleus containing erythrocytes of amphibians, fish, and birds. Within the nucleus containing red blood corpuscles of the frog and fish I was actually able to demonstrate the existence of plasmosome like inclusions as well (unpublished investigations and 1957g). This observation has recently (1959) been confirmed for the eel (*Anguilla japonica*) by N. Tsumura.

It is a well known fact that the mature red blood corpuscles are the only cells of the body of humans and of other mammals which contain no nuclei. It is a fact that they are also the only cells of the body which possess no plasmasomes (mitochondria) and no endoplasmic reticulum.

Details of the internal structure of sarcosomes are demonstrated in Figures 39 through 46, while Figure 26 demonstrates impressively regional missing of sarcosomes, between the myofibrils of the guinea pig heart (left ventricle, near the origin of the aorta).

It should also be mentioned that within the interior of sarcosomes one can frequently see dark homogenous spots which I have upon occasion designated as nucleoli of sarcosomes (Kisch 1953c, Kisch and Philpott 1953).

We should also point out the fact that as in our Figure 39 one frequently finds adjacent to sarcosomes with well developed internal lamellae sarcosomes with either a completely homogenous content or a content which looks to be crumbly. Perhaps this observed difference could be related to a different functional state occurring in adjacent sarcosomes as mentioned above or to its normal conditions.

The final proof of this author's concept about the sarcosomes and their vital importance for the heart was recently found in fresh specimens of the human heart of patients with chronic cardiac failure taken immediately after death (Kisch et al 1959, 1960a and b). A great part of the heart's sarcosomes in each one of ten investigated patients was swollen or its content degenerated into a strongly osmiophilic crumbled mass. Finally these sarcosomes disintegrated entirely and only crumbs of dark material indicate in these pathologic hearts the place between the



Figure 51 Human auricle. The arrows indicate loop shaped structures of undetermined significance lying between myofibrils whose parallel protofibrils are easily recognizable and sarcosomes which are but poorly visualized 35 000x

# X

## MEMBRANES AND OTHER STRUCTURES WITHIN THE CARDIAC MUSCLE FIBER

In addition to the sarcosomes one can recognize other formed elements within the protoplasmic matrix occupying the space between the myofibrils of the cardiac muscle and that between the myofibrils and the sarcolem. We have already made mention of some ring shaped structures (see Fig 25) (Kisch 1956e). Similar structures have been found within the human ventricle (Kisch 1960) and atrium (Fig 51). We should also make mention of the membrane which appears in pictures of sectioned tissue as bounded by two lines and which is occasionally found with such frequency that one cannot assume this structure to merely represent sectioned pieces of sarcolem (1954r). In our Figure 52 for example one can see within a region approximately  $4\ \mu$  in diameter no less than five such wavy membranes all running nearly parallel to one another. Similar lamellae can also be found within the muscle fibers of the human uricle (see Fig 51). I have in addition occasionally found branching structures of the finest order of magnitude occurring between the myofibrils of cardiac muscle. These structures are demonstrated in our Figure 53 which shows the cardiac muscle of a bat (Kisch, 1954r).

An amazing structure is illustrated in Figure 54. This figure is taken from the uricle of a guinea pig, demonstrating a row of concentrically arranged lamellae not surrounded by any common membrane lying between the sarcosomes of the uricle. This particular structure was found in the space between the sarcolem and the myofibrils (Kisch 1956r c). Small grains of the finest magnitude are seen attached to the individual lamellae. The whole structure suggests in its appearance the so called endo



Figure 53 Section through the ventricle of a bat. Myofibrils with sarcosomes interspersed between them. The arrow points to a branching canal which is running towards a myofibril. It is not certain whether this canal belongs to the endoplasmic reticulum. (From B. Kisch 1954a) 33 000×



Figure 52 : Section through the cardiac muscle of a pipe fish. The numerous membranes between the myofibrils indicated by arrows are noteworthy (From H. Kisch 1954a) 27 000X



Figure 53 Section through the ventricle of a bat. Myofibrils with sarcosomes interspersed between them. The arrow points to a branching canal which is running towards a myofibril. It is not certain whether this canal belongs to the endoplasmic reticulum. (From H. Kisch 1974a) 33 000X



Figure 54 Concentric lamellae found between the sarcosomes of a guinea pig auricle. Tiny bubbles are seen adherent to the lamellae. (From B Kisch 1956)  $\times 47\,000$

plasmic reticulum which is found in other tissues (see Palade 1956)

Again this entire field requires extensive further investigations. The endoplasmic reticulum found in the muscle fibers as well as in other organs of the body is not at all clarified in regard to function as yet. As a matter of fact it has not yet been proven that the endoplasmic reticulum is in reality a true reticulum that is a real web structure. It might perhaps be more



Figure 55 Atrium of the cow's heart Sarcosomes between myofibrils C = Cytoplasmic material ER = Profiles of endoplasmic reticulum 12 000×

correct to designate the structure as an ultramicroscopic system of canals of changing outline and content. The question has recently been raised as to whether this so called "reticulum" is perhaps identical with the ergastoplasm of earlier histology. Also the possible relation of the reticulum to the so called Golgi apparatus has been indistinctly discussed at various times (Dalton and Leitz 1956 Palade 1956).

I would like to confine myself here to the photographic demonstration of membranes and rods or lamellar structures as



they have been observed up to now within cardiac muscle, while avoiding theoretical speculation as to the significance of these structures. We need to stress only one point: these well delineated structures are definitely not artifacts but rather they are naturally occurring ultramicroscopic structures in the heart and they occur in human cardiac muscle as well (see Fig 51). Probably they are not identical to those diffuse accumulations of liquid which were designated as lacunes, cisterns, etc. Whether in life all of these spaces are also well demarcated by membranes as they are in the electron microscopic image and especially in diagrams should not be decided as yet. In my opinion the lacunes and the entire so called endoplasmic reticulum are a liquid part of the cytoplasm having fluctuating boundaries and continuously changing contents differentiated from other parts of the cytoplasm probably by physical, chemical and physico-chemical properties. The time has not yet arrived to attribute a physiological function to such structures as so little is known about them. It should only be emphasized that the mature erythrocytes of mammals are probably the *only cells* of the body which contain no nucleus, no plasmasomes (mitochondria), and no endoplasmic reticulum as I have been able to demonstrate. (See page 112 for the endoplasmic system of channels in Purkinje fibers.)

A possible relationship between but not identity of endoplasmic reticulum and finest droplets present in endothelial cells of capillaries in muscle fibers of the heart, in nerve axons and in other cells should be considered. They probably play an important role in the exchange of material between the cells and the intercellular space (see our Fig 78 from the human ventricle). Warren Lewis called (1931) the drinking of droplets by cells pinocytosis. H. Stanley Bennet has discussed droplets as a type of Membrane flow (1956). I call this droplet transfer dropletolysis in contradistinction to molecular transfer.

Whatever one's opinion may be concerning these and other ultramicroscopic membranes, lacunes and droplets, it seems (1952b, c) that electrophysiologists must of necessity take serious cognizance of all these morphological entities when formulating their

own explanations attempting to make use of Bernstein's membrane theory. It is poor practice to dispose of factual organic entities by means of a few words. In reality where within the heart is this membrane of which the theoreticians of electrophysiology tell us? Is it perhaps the surface of a group of musclefibers or the sarcolem or even perhaps the surface of the myofibril which although it is not an organic membrane represents an interphase (*Phasengrenzschicht*)? What role if any do the above mentioned various intrafibrous membranes play in the electrophysiology of the heart and what roles are played by the membranes which surround the sarcosomes and lastly, by the Z bands which traverse the muscle fibers?

Only recently does this consideration which the author has emphasized since 1950 seem also to be shared by others (Moore and Ruska 1957).

# XI

## INTERCALATED DISCS

Cardiac muscle is transected perpendicularly to its long axis by fine striations, which are partially arranged in step wise fashion and which partially transect the muscle in a straight line. These striations are known as intercalated discs in the English literature. In the German literature they are referred to as *Glanzstreifen*, *Kittlinien* or *Schaltstücke*. In the French literature they are referred to as *bandes scalariformes*. The width of these discs and the frequency of their occurrence varies with the species of animals studied with the age of the animal and within the same heart with the area sectioned. Apparently, they are found particularly frequently in older individuals in the papillary musculature and in the left ventricle by contrast they are found less frequently in the auricles.

Since the time (1951b) of the first attempt to demonstrate intercalated discs electron microscopically, various studies into the ultrastructure of these entities have appeared. In 1953 V. L. van Breemen investigated the intercalated discs of the dog heart and concluded that the electron microscopic pictures of his studies substantiated the theory repeatedly stressed in the light microscopic literature that the intercalated discs represented the boundary between two neighboring cardiac muscle cells. He considers it significant to this concept that those pictures which are designated as representing a section through an intercalated disc all contain a line which is in direct continuity with the sarcolem which line represents the boundary between individual cardiac muscle fibers as well. Van Breemen also emphasizes the fact that in his experience the myofibrils of the muscle fiber never traverse the intercalated discs. Rather the myofibrils end at the discs and

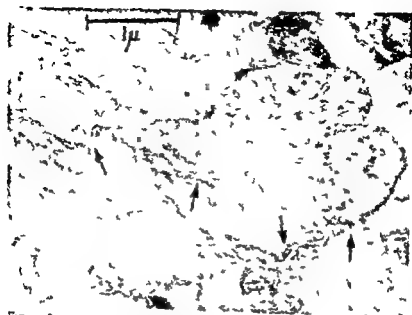


Figure 56 Auricle of the mouse Arrow points to what is probably part of an intercalated disc 25 000x

therefore the intercalated discs do not represent an intracellular structure. He regards the intercalated discs as a corrugated separator between the muscle cells made up of two layers of membrane which in turn represent the boundaries of two adjacent heart muscle fibers. A paper by Poche and Lindner (1955) agrees almost completely with the findings and the opinions of van Bree as does a study by Sjostrand and Anderson (1954) Kisch (1956a) Lindner (1957) Moore and Ruska (1957) Muir (1957) and Lanzavecchia (1957).

The investigated animals included mammals frogs and chicken embryos.

The present work contains two illustrations (Figs 56 and 57) in which similar membranes running in zigzag fashion are seen in the auricle and ventricle of the guinea pig. I have found membranes of very similar form in freshly fixated human auricular tissue and in the heart of the pipefish (1954a). Despite this it would seem necessary to continue these investigations further before one can wholeheartedly endorse the interpretation given to

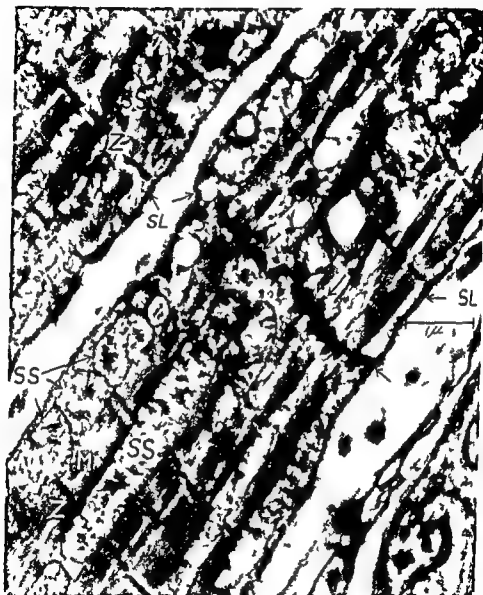


Figure 57 Left ventricle of the guinea pig heart. A zigzag line cuts directly across a muscle fiber and is connected with the sarcolemma SL. The myofibrils with their characteristic striations and the sarcosomes SS are easily visible. The myofibrils do not seem to be interrupted by the perpendicular line. 17 000 $\times$ .



Figure 58 Intercalated disc (↑) in the cow's atrium 13 000X

all these zigzag lines by the above mentioned authors. It should be noted however that in all of my corresponding pictures one can also see a clear connection between the zigzag lines and the sarcolem. This would seem to strengthen the above author's theory that the intercalated discs represent cell boundaries.

In contrast however I would like particularly to call attention to Figure 57. Should the strongly osmiophilic zigzag line pointed out by arrows in this figure actually represent an intercalated disc then there can be no doubt that the individual myofibrils are not entirely interrupted by this line which connects with the sarcolem. Rather the myofibrils continue across the line and beyond.

In recent investigations the same type of intercalated discs have been found in Purkinje's fibers of the ventricle (not yet published own observations).

Another remark seems to be in place here. Recently the ultra-microscopic structure of the intercalated discs and their connection with the sarcolem has been used as proof that the heart muscle is not a syncytium (Poche and Lindner 1955 Lindner 1957 Muir 1957).

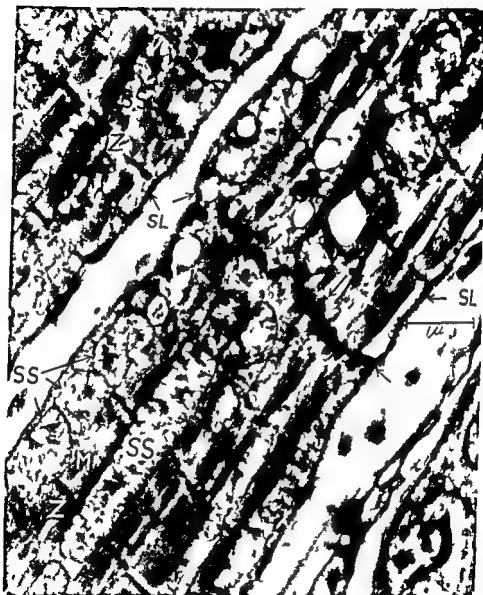


Figure 57 : Left ventricle of the guinea pig heart. A zigzag line cuts directly across a muscle fiber and is connected with the sarcoplasm SL. The myofibrils with their characteristic striations and the sarcosomes SS are easily visible. The myofibrils do not seem to be interrupted by the perpendicular line. 17 000 $\times$

## XII

### THE NUCLEI

Light microscopy has known for a long time that the nuclei of the cardiac muscle fiber are predominantly located in a central position. This is in contrast to skeletal muscle where the nuclei are predominantly located towards the periphery that is in close proximity to the sarcolem. The number of nuclei found in the embryonic heart is markedly in excess of the number found in the heart of the adult animal. This observation previously made in the mouse (Kisch 1951) can now be confirmed in the case of the guinea pig and the human (author's unpublished studies).

In the ventricle the nuclei of the muscle cells assume as a rule an elongated ellipsoid form with the long axis running parallel to the myofibrils. Whenever the nuclei of ventricular musculature appear round in a photograph the most probable explanation is that the photograph represents a cross section through a nucleus rather than that the nucleus is in actuality spherical. Very frequently the nuclei appear to have a bizarre form (Figs 60, 61 and 62). Sometimes they also seem to be deeply indented. If a section cuts across such an indentation or several of them the resulting picture may seem to show one large nucleus and in addition one or more smaller nuclei (Fig 62) whereas in reality we are dealing here only with one or more additional pieces of the same nucleus (Figs 59 and 62). As repeatedly pointed out since 1951 one always finds a collection of sarcosomes in close proximity to the nucleus of cardiac muscle and a collection of plasma somes in close proximity to each nucleus in the endothelium of blood vessels.

Since it has been shown that the function of the sarcosome is to provide for the tissues an available source of necessary enzymes



This author has emphasized repeatedly since 1950 that the ultra-microscopic inner structure of the muscle fiber and the presence of its sarcolem excludes the concept of its being a syncytium (1951, p 25 1952c 1957g and so on)

Concerning intercalated discs in the human ventricle see Chapter XIX (Fig 95)

## XII

### THE NUCLEI

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Concerning intercalated discs in the human ventricle see Chapter XIX (Fig 95)



Figure 61 Nucleus from a Purkinje fiber of the cow's heart. Note the spongy structure of the three nucleoli. 14 000X

for chemical exchange the above observation is an important indicator that *the nucleus even when it is not dividing has a constant stable rate of metabolism* (Kisch 1951)

Every nucleus exhibits a thickened outer bordering layer the nuclear membrane and one or more nucleoli. The nuclear membrane often has chromatin fragments attached to its inner surface. Perhaps these chromatin fragments represent post mortem or necrobiotic phenomena as they rarely occur in satisfactorily fixed nuclei with uniformly distributed contents. Because of the ultra thin sections made possible by today's technology we now occasionally see pictures of nuclei depending on the plane of sectioning which contain no nucleoli at all (Figs 59 and 62). With

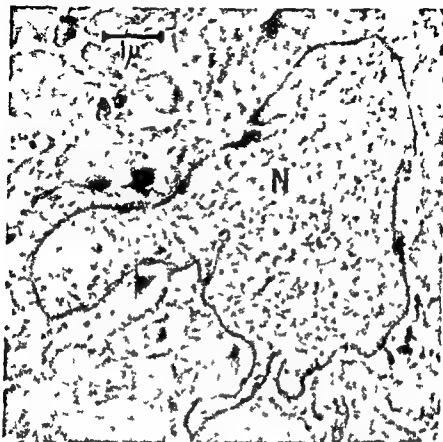


Figure 59 Bizarre form of the nucleus in a human ventricle 13 000X

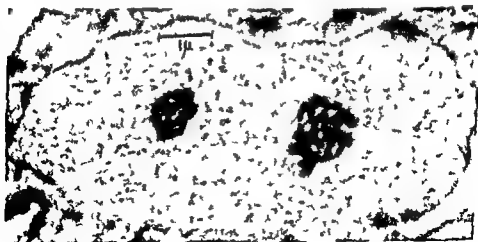


Figure 60 Nucleus from the ventricle of a guinea pig. Note the spongy appearance of both nucleoli 13 000X

fibrils. At other times masses of sarcosomes crowd in between the nucleus and the myofibrils. Occasionally one sees the nucleus lying in a wide environment of mytoplasm (endoplasm) surrounded by many sarcosomes. We have not been able as yet to demonstrate any typical differences between the nuclei of the auricles and those of the ventricles. However it would seem that the elongated ellipsoidal nuclear shape is more prevalent in the muscle fibers of the ventricle whereas the auricles not infrequently possess the more bizarrely shaped type of muscle nuclei (Fig 59).



Figure 62 Deeply indented nucleus from the human heart surrounded by sarcosomes (SS)

thicker sectioning one rarely sees nuclei in the heart muscle which do not contain at least one and usually several nuclear inclusion bodies. These bodies are not merely solid spots dark through their ability to absorb osmium stain but rather *the nucleoli exhibit very often a coarse sponge like inner structure of their own* (Figs 60 and 61). The rest of the nuclear content present in appearance of more or less homogeneous material or granulation depending on the quality of fixation and upon other processing of the tissue. Sometimes the nucleus is picked tightly between muscle



Figure 63 Interstitial cell in guinea pig heart. Note the long thin extension of the cytoplasm running along the sarcolem of a neighboring muscle fiber. (From H. Kisch 1936a) 8 000×



Figure 64 Human auricle. A cell found between muscle fibers. The protoplasm is filled with tiny strongly osmophilic droplets giving the entire cell an appearance of having been sprayed with confetti. 17 000×



## XIII

### INTERSTITIAL CELLS

In the space between the individual muscle fibers of the heart we find vessels, nerves and certain types of cells which shall be discussed now.

The first type of cells found here are the pericytes or Rouget's cells which in part are found directly adjacent to the capillaries. These cells will be the subject of a later chapter (see p. 128 ff). In addition one finds cells in the space between the muscle fibers of the heart which most probably belong in the category of connective tissue cells. These interstitial cells stretch by means of wing shaped or lamella shaped extensions between the sarcolem of adjacent muscle fibers. They have large nuclei and by comparison a scanty compact quantity of protoplasm with far reaching lamella shaped extensions. Plasmisomes are found within their protoplasm in proximity to the nucleus. In the electron microscopic picture of ultra thin sections the fine lamella shaped extensions of these cells have an appearance identical to sections through membranes which extend between two individual muscle fibers. Only in uteris where as in our Figure 63 the connection between these membranes and the protoplasm of the interstitial cell can be shown does the true identity of these interfibrous membranes become clear. Such membranes can also surround entire groups of cardiac muscle fibers in which case they may become what in an earlier chapter (Figs. 5 and 6) was referred to as a sarcolem of the second order. These facts can be best seen in the ultramicroscopy of teased tissue preparations.

Louw (1953) has demonstrated for epithelial cells of the lung alveolus that the extensions of these cells can run in an unimaginably thin layer along the surface of alveoli covering the capil-



Figure 63: Interstitial cell in guinea pig heart. Note the long, thin extension of the cytoplasm running along the sarcolemma of a neighboring muscle fiber. (From B. Kisch, 1958a.) 8,000X

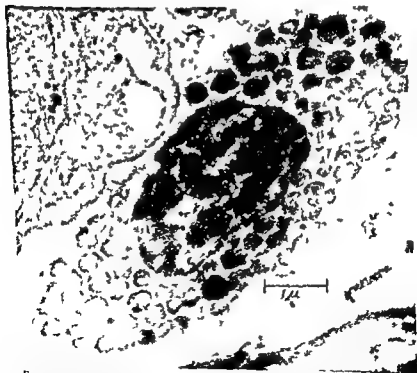


Figure 64: Human muscle. A cell found between muscle fibers. The protoplasm is filled with tiny, strongly osmophilic droplets giving the entire cell an appearance of having been sprayed with confetti. 17,000X

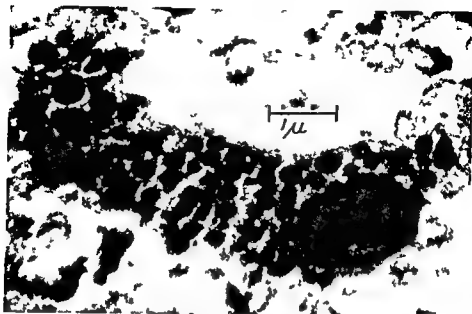


Figure 65 A cell similar to the one shown in Figure 64. This cell was obtained from sections through the aortic valve of another human patient suffering from rheumatic valvular heart disease. 15 000 $\times$

larities. Similarly, fine extensions of the interstitial cells stretch, as I have been able to show, between the individual cardiac muscle fibers as well (1956a, 1957g; see also Fig. 63). This particular arrangement undoubtedly has physiological significance in the cardiac muscle, although at the present time this significance is still unknown to us.

These observations in the lung and in cardiac muscle make it requisite that histologists and physiologists become aware of the fact that the individual cells in the tissue of a living organism are not simply compact masses of closely grouped entities (nucleus with surrounding protoplasm). Rather the protoplasm of these units is in many cases connected with the space in between the neighboring cells by means of pseudopod extensions. Light microscopy dependent as it is upon the use of stains is not able to demonstrate for us properly these findings which seem significant to an understanding of the positioning of the individual cell within a tissue. I was able to show similar conditions to exist in endothelial cells of the smallest ducts of the lungs (1958b).

The above observations also help us to understand the structure

of the simplest capillaries of which we will speak in more detail in a later chapter. Lamella shaped extensions of the endothelial cells which make up the walls of the simplest capillaries expand and surround the capillary blood stream in a tube like manner. In those places where the extensions finally meet and overlap in the periphery, there arise the mural slits of the capillary tube which I have repeatedly described (1955a, 1956b) and will describe further in the next chapter.

I would still like to mention one special type of cell which I have occasionally found between the cardiac muscle fibers. In preparations of the dilated auricle of two human hearts containing old rheumatic valvular defects I found a unique type of cell whose entire protoplasm was studded with osmiophilic drops of finest dimension (see Figs. 64 and 65). These osmiophilic drops exhibit no internal structure which might allow them to be labeled as plasmosomes. They are rather homogeneous ultra microscopic droplets made up of lipid or some other osmiophilic matter. I cannot at the present time say whether these cells are a pathological manifestation or not. Neighboring cells within the tissue of these two hearts did not exhibit the unique traits enumerated above. I have occasionally seen large quantities of similar osmiophilic droplets in the endothelial cells of the auricle of the bat. In these bats as in the humans the appearance of the osmiophilic droplets was restricted solely to one type of cell while neighboring cells in the deeper cell layers contained no droplets.

Interstitial cells of another kind are found near capillaries in the human ventricle (Kisch 1960b). They contain many ghosts of mitochondria and an extremely widened endoplasmic reticulum. Their appearance reminds one of plasma cells. See such an L.R. cell Chapter XIV (Fig. 97 and 95).

## XIV

### THE ENDOCARDIAL CELLS

The endothelial cells of the inner surface of the heart have not yet been extensively investigated with the electron microscope. Some isolated observations on the aortic valve of the guinea pig have already been published (1956a). More recent observations are in agreement with these earlier findings. As seen in Figure 66 the endocardial cells have large nuclei and their basilar membrane bounds on a layer of very fine granular material which is in direct contact with the sarcolem of the outermost muscle fibers. I called it the sub endothelial or sub endocardial stratum.

One is struck with the fact that certain of these endothelial cells especially in the embryonic heart are extremely rich in plasmalomes. Since one can generally demonstrate that tissues with active chemical exchange are also rich in plasmalomes the above observation would seem to indicate that the endothelial cells of the heart possess a very active metabolism and therefore most probably have an important although as yet undetermined function. We are at present engaged in a special study of this component of cardiac muscle.

The many unmyelinated nerve axons which can be found in the subendothelial stratum between endothelium of the heart and sarcolem are probably the afferent branches of the Bezold Jarisch reflexes (Kisch 1956b 1957g). Their existence has recently been confirmed by electron microscope pictures of the atrium of the turtle (Fawcett and Selby 1956).



Figure 66 Endothelial cells from the auricle of a guinea pig. These cells containing large nuclei have their basal membranes and cell boundaries indicated by arrows. Beneath them there is a finely granulated layer the sub-endothelial stratum with inclusions the latter indicated by an X. These inclusions represent nerve axons. The granulated layer bounds directly on the sarcolem (SL) of the outermost muscle fiber. (From B. Kisch 1956.)  
10 000x

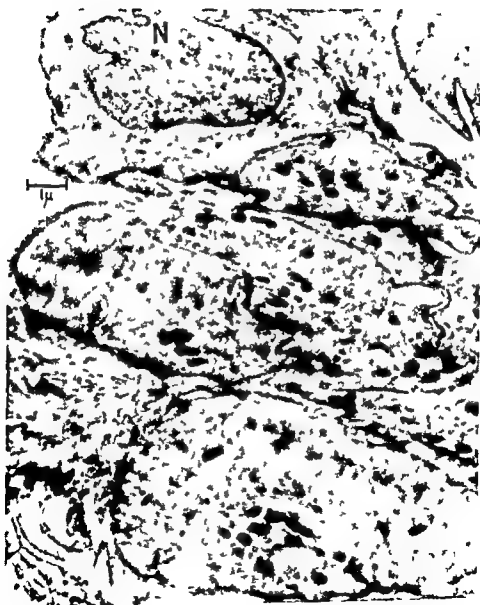


Figure 67 Endocardial cells from the ventricle of the rabbit. Big nuclei and scanty plasmisomes. 8 000 $\times$



Figure 68 Endocardial cells from the heart of a cow (ventricle) Big nuclei with plasmasomes in their neighborhood and profiles of  $r$  system of smooth channels 9 000 $\times$



## XV

### THE CONDUCTIVE SYSTEM (PURKINJE FIBERS)

The history of the discovery of the stimulus conducting system in the heart can be divided into two phases. In the early Nineteenth century (1846) the Breslau physiologist, J. E. Purkinje discovered within the ventricles the subendocardial system of specific fibers, which still today bear his name.

Purkinje of course did not yet realize the physiological significance of this structure. At the end of the Nineteenth Century (1893), W. His, Jr. discovered the muscle bundle which connects the auricles with the ventricles and recognized the physiological function of this structure generally referred to as the Bundle of His. L. Ashoff and his collaborators, particularly Tawara (1906) then studied the ramifications of the conductive system (Tawara's Bundle Branches) whose finest subdivisions are Purkinje's fibers.

Textbooks of histology usually mention the bundle of His and its branches only as a kind of succulent muscle fiber rich in protoplasm.

It seemed enticing to study this kind of tissue with the electron microscope.

As material, the author used Purkinje's fibers and the chordae tendineae spinae of cattle and rabbits especially the ribbon traversing the right ventricle (moderator band).

These investigations of the conductive system which are by no means as yet completed have thus far yielded the following results.

Whenever one of the smallest peripheral trabeculae also known as Purkinje's fibers or one of the bigger false chordae tendineae is fixated in 1% Osmium Ringer's solution one can distinguish a strongly osmiophilic inner thread like the wick in a candle surrounded by a much less osmiophilic enveloping mantle.

On their surface all the above mentioned false chordae or trabeculae containing Purkinje's fibers are covered by the endocardium (endothelial cells). Inside as is well known they are very rich in sarcoplasm and poor in muscular elements. The latter individual myofibrils are very similar to every other myofibril. Their protofibrils are recognizable and the different zones such as Z M I H exhibit their usual significant differences. The Z-bands which in the myocardium connect the individual myofibrils at the level of the Z-zones seem to be entirely missing in Purkinje's fibers. In the heart muscle the Z bands are connected with the sarcolem but as yet I have not been able to find the typical garland type of sarcolem connected with Z-bands. In these trabeculae there is rather a straight double lined sarcolem.

Very often one finds a single myofibril surrounded by a large amount of sarcoplasm and sometimes two or three myofibrils are found lying close together. The familiar picture of masses of myofibrils lying parallel to each other separated by long rows of sarcosomes as can be seen in our different figures of the heart muscle does not exist here apparently. One gets the impression that Purkinje's fibers in the false chordae simply do not contain normal myocardial muscle fibers of normal structure separated from each other by great amounts of sarcoplasm but rather that Purkinje's fibers contain within their sarcolem many individual myofibrils distributed within a great amount of sarcoplasm. Such muscle fibers are concentrated in the central osmoplutic cylinder of the big false chordae and of the small endocardial trabeculae of the heart. The number of sarcosomes found between the myofibrils seems less than that present in the muscle fibers of the myocardium of the same animal. They have a membrane and contain an inner laminar structure and also occasionally small vacuoles. This smaller amount of sarcosomes in the conductive system of cattle may be as emphasized before connected with the observed fact that the conductive system is more easily exhausted under conditions of starvation or lack of oxygen than is the myocardium. However not only the conductive system but the myocardium of cattle too contains less sarcosomes than that of small animals and that of man (see p 66). Most striking is the very large number of finest vessels, channels and lacunae of every size found



Figure 69 From the finest trabecules in the ventricle of a cow containing isolated myofibrils (Purkinje fibers) in a great amount of cytoplasm. No Z bands can be seen but different sarcosomes and the Z zones of the myofibrils  
12 000×

within the cytoplasm of Purkinje's fibers and between them. This astonishing finding leads to the conclusion that this system of vacuoles and channels which may correspond to the endoplasmic reticulum in other cells is in some way involved in the great capacity for quick stimulus conduction of Purkinje's fibers. Larger concentrations of plasmisomes (mitochondria) are often located just adjacent to the channels of this system. In line with the fact emphasized above that the enzyme bearing plasmisomes are



Figure 70 Purkinje fibers of the heart of a cow. The sarcosomes (SS) are very scanty and the system of small channels (↑) in the cytoplasm abundantly developed. Notice also like in the previous figure the presence of the fibrillar zones Z, M, H, I. 16 000 $\times$ .

always aggregated near organelles with a high rate of metabolism such as the muscle fibrils in the myocardium or the nuclei in the heart and in the capillaries. This accumulation of plasmasomes would speak in favor of a high metabolism in this system of channels. That suggests again their having a significant function in stimulus conduction.

From the viewpoint of the physiologist one should notice that there is no difference histologically between the myofibrils of the myocardium and the myofibrils of Purkinje fibers. Therefore it is not understandable why these fibrils should conduct a stimulus much quicker than do those of the myocardium but physiological research has proven that the stimulus is conducted much quicker in Purkinje's fibers than in the myocardium. This suggests that it

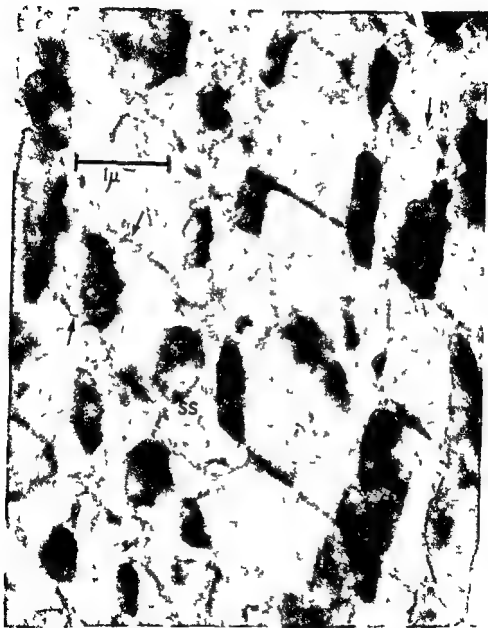


Figure 71 Questionable Purkinje fiber of rabbit (moderator band) Much cytoplasm between the individual myofibrils also sarcosomes also by arrows indicated vesicles 20 000X



Figure 72 A group of protoaxons surrounded by a Schwann's sheath from a very small trabecle containing Purkinje fibers in the cow's heart 18 000X

is the substance found in the muscle fiber between the myofibrils and not the myofibrils which determines speed of conductivity. Perhaps it is also responsible for stimulus conduction in general. In this regard there is a definite difference between the muscle fiber of the myocardium and that of the conductive tissue. The cytoplasm between the myofibrils in the latter is much more abundant and the spaces of endoplasmic reticulum, lianes and cisterns of liquid are much bigger and more plentiful than in the myocardium (Figs 69, 70 and 71). The concept that this part of Purkinje's fibers and the entire conductive system serves a specific function is well compatible with a similar opinion of H. Rusca *et al* (1955) about the endoplasmic reticulum.

Nuclei can be also found in Purkinje fibers and occasionally as seen in our figure one finds vessels which represent capillaries or finest lymph vessels. Nerve axons are also present even in the smallest trabecules of the ventricles (see Fig 72).

It is well known from light microscopy that the trabeculae and the false chordae in which Purkinje's fibers are enclosed are extremely rich in lymphatic vessels. It may well be that all the

mentioned very small vesicles, canals and lacunes or cisterns, which can be seen in Purkinje's fibers at the finest ramifications of lymphatic vessels and not part of an endoplasmic reticulum. Nowhere can one find the small particles attached to these canals which Palade found to be typical satellites of the endoplasmic reticulum (1958). Of course where is the strict borderline between endoplasmic reticulum and the finest ramifications of lymphatic vessels? The location alone (intercellular vs intracellular) is significant but still may not be decisive. In distinction to the protoerythrales (see next chapter) whose wall always consists of an endothelial cell the profiles of the system of canals and lacunes in our pictures of Purkinje's fibers never show a surrounding endothelial cell but always an easily recognizable membrane only. The big lymphatic vessels outside the muscle fibers however are tubes built out of endothelial cells, similar to the erythrales.

In the moderator band of the rabbit we have found many muscle fibers exactly resembling normal myocardial fibers of the same animal, with great masses of sarcosomes. We have also found there another type of muscle fiber with isolated myofibrils embedded in a large amount of cytoplasm and fewer though still many, sarcosomes.

It is known (Monckeberg 1908) that some of the spurious chordae of the heart contain only Purkinje's fibers some only regular myocardial muscle fibers and some contain both. The last seems to be the case in the moderator band of the rabbit. Inter-calated discs of the same appearance as in the myocardium have been found in Purkinje fibers also.

In the chordae spuriae are found many capillaries and individual nerve axons as well as smallest nerve fibers. It is noteworthy that a structure like the rabbit's moderator band traversing the right ventricle freely and having a diameter of only a fraction of a millimeter is not supplied with blood only from the content of the right ventricle via the surface but rather from innumerable capillaries as well. This factor points to the importance of an abundant blood supply to Purkinje fibers in the moderator band and to the physiological importance of its normal function.

## THE CAPILLARIES

## A. NORMAL CAPILLARIES

Early in my investigations I had assumed on the basis of the electron microscopic pictures that some portion of the capillaries of cardiac muscle did not merely run between the muscle fibers but rather penetrated into these fibers. This opinion had seemed to be borne out in the examination of stained light microscopic preparations. With the development of electron microscopic technique and more especially in studying the ultramicroscopic teased tissue preparations we have recently been consistently able to demonstrate a fine membrane, the sarcolem, between the capillary wall and the muscle fibrils. Assuming that this fine membrane always represents an actual muscle fiber boundary, it would seem appropriate to assert, at least until it can be proved otherwise, that capillaries in the myocardium run in spaces between muscle fibers (1956b) and not within the muscle fiber proper.

In the text that follows when we speak of the structure of the capillary wall we will have reference exclusively to the capillaries of heart (auricle as well as ventricle) and of skeletal muscle. It is a well known fact that the capillaries of other organs such as the liver or the kidney exhibit unique peculiarities of structure (Krogh). Even the capillaries of the lung have been shown to possess certain peculiarities of structure and it should therefore be stressed that the capillaries under discussion in this section are only those found in muscle tissue.

One can safely estimate the thickness of the wall of the most primitive capillaries at between 0.2 and 0.5  $\mu$ . This is the case except in places where the sections contain a nucleus or the cytoplasmic cushion in which the nucleus is embedded. At such places the wall of the capillary may have a diameter of 1  $\mu$  or more. This fact can also be demonstrated by examining serial sections.



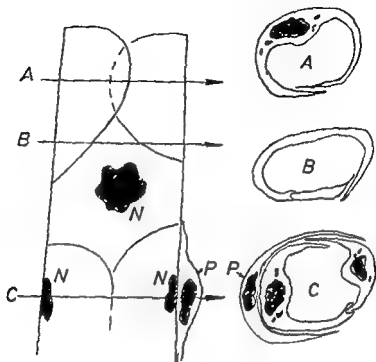


Figure 73 Diagrammatic representation of the simplest type of capillary (protocapillary). The three schematic section planes a, b and c illustrate the various possibilities as to appearance in the electron microscopic picture which such a protocapillary can present. All sections are assumed to have passed directly perpendicular to the long axis of the capillary. In oblique sections the possible appearances are of course much more varied still. N is the nucleus. P is a pericyte (Rouget cell) surrounding the capillary tube with its cytoplasm.

When they are not totally collapsed (Figs 84 and 85) the simplest capillaries viewed through the electron microscope exhibit one of three forms depending on the plane of sectioning. The first form assumed by the capillary is that of a more or less perfect circle surrounding a lightly granulated surface. The second form if sectioned obliquely is that of an ellipse and the third form seen when the plane of section cuts the capillary through its long axis is that of two ribbon shaped structures running parallel to one another. The finely granulated area surrounded by the capillary wall is the space which in life is occupied by the blood as it flows through the capillary. In this space one frequently

sees sections of red or white blood corpuscles (see Figs 73 78 79 etc) or thrombocytes

The simplest type of capillary is a tube consisting of a sequence of single endothelial cells only. This I have called a *protocapillary*. A section through such a capillary exhibits the clarity and simplicity of structure of the capillary wall. It would seem incidentally that the thickness of this wall depends in part on the extent of filling of the capillary itself (A Krogh 1936 Kisch 1954c 1956b).

Two types of capillaries can be distinguished. One which I call the *protocapillary* is a sequence of irregularly shaped endothelial cells with a widely expanded cytoplasm each of which at least in some places envelops entirely the capillary blood stream. Figure 73 shows in a diagram this situation and the electron microscopic appearance of different sections through the *protocapillaries* in the heart muscle, auricles and ventricles. Electron microscopy was able to reveal the enormous amount of such *protocapillaries* in the heart.

Wherever the diameter of the capillary blood stream is too big to permit a single endothelial cell to embrace it completely, two or more such cells will be needed to build the capillary tube. The histological picture of the capillary wall will then present that of a mosaic of endothelial cells familiar from light microscopy. In cross sections there never will be only one slit in the capillary wall but at least two usually even more and the cytoplasm of these capillaries is no longer so widely extended as in *protocapillaries* and this results in a thicker wall than in *protocapillaries*. In this consideration there is mention made only of the endothelial cells.

*Pericytes* surround *protocapillaries* as well as capillaries with mosaic endothelium. They become more frequent and smooth muscle cells are added when the pre- or post capillary stage is reached.

The capillary regularly exhibits an outer fairly regular membrane (*membrana externa*). The thickness of this membrane if double lined is approximately 300 Å. It is the boundary between the capillary and the surrounding tissue and may be regarded as the basal membrane of the endothelial cell. This simplest type of capillary is made up of a sequence of single endothelial cells

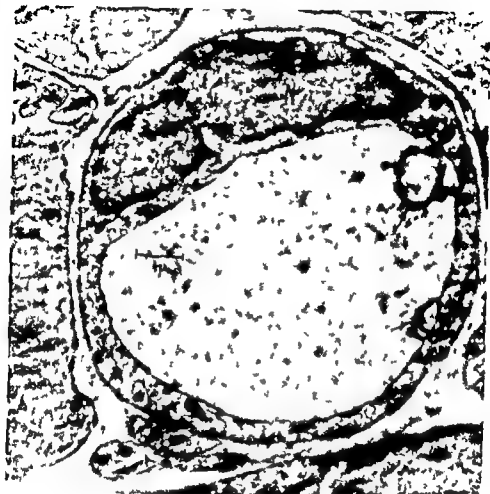


Figure 74 Protocapillary from the right auricle of a guinea pig. An arrow indicates the slit in the capillary wall; the other arrow the endothelial tentacles or villi. The nucleus N, plasmisomes near to it, and profiles of finest channels in the cytoplasm can be seen. 11 000X

adjacent to one another. The line which separates the capillary wall from the content of the capillary (*membrana interna*) always is seen in electron microscope pictures as a single line. This line does not run exactly parallel to the *membrana externa* but rather it pursues an irregular, scalloped course. The *membrana interna* exhibits also fine processes which extend like pseudopods or fringes into the lumen of the capillary. I called them the endothelial villi or tentacles of the capillaries.

One is especially struck by these extremely fine, long (in longi-



Figure 70 Capillary of the human ventricle In the upper part the cytoplasm must be sectioned near the nucleus as suggested by its diameter in this place and the presence of plasmasomes (P) which are usually found near to the nucleus The arrow indicates the slit in the capillary V an endothelial tentacle PE part of a pericyte (Rouget's cells) A a neighboring nerve axon The arrows in the cytoplasm hint to profiles of a system of fine channels (Endoplasmic reticulum) 12 000X

tudinal sections) thread like extensions reaching from the endothelial wall into the lumen of the capillary (see Figs 74 75 77) As sections sometimes cut these extensions through their entire length it might be more correct to regard them as thin ribbons rather than as hair like cilia It is only in longitudinal sections that these extensions appear as cilia In some pictures one sees



Figure 76 : Longitudinal section through capillary in the ventricle of a mouse. N endothelial nucleus. The arrows point to two slits in the wall the crosses to profiles of a system of small channels in the cytoplasm. No cement exists between the endothelial cells. 13 000 $\times$

them as separated pieces lying within the capillary content cut off at their bases. This appearance is illustrated in our Figure 83 designated by a star. Such extensions are frequently found at the slits within the capillary wall that will be discussed later on. They measure approximately 300 to 800 Å in diameter and they may be several microns in length. Our Figures 74 and 75 show them in animal capillaries as well as in human ones.

The two membranes discussed above, the inner of which is more of the type of an interphase than a true membrane, enclose between them the protoplasm of the endothelial cells and form together with this protoplasm the capillary wall. This fact would lead one to suppose, and indeed our figures bear this out, that the capillary wall does not have a uniform thickness throughout. It has, for example, been mentioned that at the places where the nucleus together with its plasmisomes is found to lie between the membrana externa and the membrana interna, the thickness of the capillary wall at that place where the nucleus has its maximum diameter, will approach 2 to 3  $\mu$  or even more. In contrast in

areas far removed from the nucleus where there is very little protoplasm near the *membrana externa* the diameter of the capillary wall is found to be only about 300 Å.

In this type of investigation one is again and again struck by the fact that the heart (auricle and ventricle and even the moderator band) contains a far greater quantity of capillaries than does the skeletal musculature of the same animal.

The protoplasmic mass between the two bounding membranes of the capillary wall contains nuclei, plasmosomes, fine granules and vacuoles all in a uniform connecting medium. The protoplasm also contains an ultramicroscopic system of canals similar to that which is designated as endoplasmic reticulum in the cytoplasm of other cells (Kisch 1957b, 1957g; Moore and Ruska 1957h) and many smallest droplets.

From the physiologic point of view all of the above signifies that, thanks to the corrugated contour of the *membrana interna* with its extensions and tentacles the inner surface of the capillary wall must be far greater still than our present estimates (Krogh) would lead us to believe. Perhaps this architecture of the inner surface of the capillary wall may contribute to a slowing down of the peripheral blood flow within the capillary. Perhaps the long processes extending into the blood stream play some part in clot formation. Our figures also point out that it is not correct to regard the capillary wall simply as an extremely thin permeable or semipermeable membrane. Rather it is a complicated protoplasmic living system whose active selective function may play a key role in the permeability of the capillary wall. Finally, one can easily imagine (Kisch 1955a, b, 1956b, 1957g) that a thickening of the capillary wall under pathological conditions to twice its normal diameter a phenomenon which would completely elude the light microscopist would present an insurmountable barrier to the gas and other chemical exchanges which normally take place in the area of the capillary. This thought poses a whole new series of problems and opens new vistas to the field of circulatory pathophysiology and so do the droplets in the capillary wall.

In capillaries whose walls are made up of a single endothelial cell the boundary between their adjacent margins can present one of several appearances. Most often the cells will overlap in the

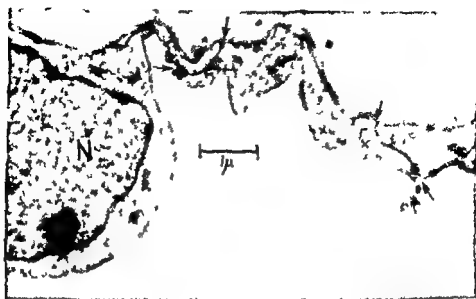


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Figure 78 Capillary from the human ventricle C Lumen of capillary Sl Sarcolem M Myofibril SS Sarcosome Many droplets in the capillary wall and at the inside of sarcolemmata. Arrows indicate where droplets empty into intercellular space (Droplet transfer Droplet-dialysis) 40 000 $\times$

It has been known for a long time that capillaries whose walls are made up of a single endothelial cell layer are not constructed in such a manner that cells of cylindric form and parallel base lines are positioned with their borders touching one another to form a long cylindric tube. Rather the cells are arranged in a spiral fashion (Benninghoff 1930) corresponding to their polygonal or rhomboid shape (Krogh). Because of this phenomenon a section transecting a capillary at an angle perpendicular to its long axis does not necessarily, although it may produce a picture of a ring





Figure 77 A piece of a capillary from the human uricle showing the origin of one of the thread shaped extensions of the endothelial cells  
46 000x

manner of a rolled up piece of paper leaving a diagonal slit between the borders (see diagram Fig 73). In other cases, the borders will fit tightly with close indentations or will intermesh to form a joint (dovetail or zigzag) (H Ruska 1954 Kisch 1955a b d, 1956d 1957g Palade 1956). In each of these cases the final result is a slit or a split which connects the inside of the capillary to the outside. As yet I have never found special material to be contained within these mural slits. The electron microscopic pictures seem to exclude the existence of any kind of cement within such slits (1957g). These slits in the capillary wall could well serve to facilitate exchanges into and out of the capillary. One frequently finds the above discussed endocapillary extensions (endothelial villi) reaching into the capillary blood stream exactly at the site of these slits in the capillary wall.

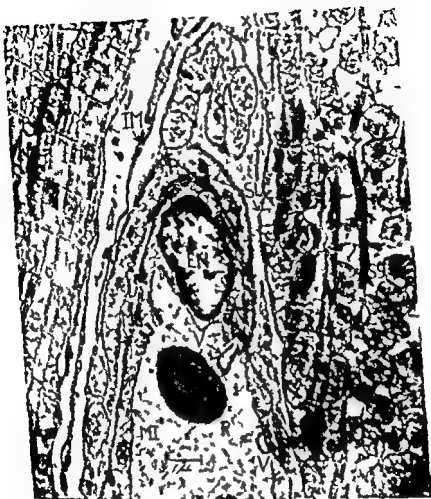


Figure 79 Capillary from the left ventricle of the guinea pig heart. A pericyte (P) is seen to be sitting like a cap over the capillary in the region of the endothelial cell nucleus (FN). The structure directly above the pericyte (X) presents a group of nerve axons. II contains as do similar pictures not published in this volume longitudinally and transversely sectioned fibrils of the finest diameter. To the left there is seen an extension shaped like a membrane (IM) surrounding the capillary. Its connection with the cell body of an interstitial cell can be seen above. To the right and to the left of the capillary there is a muscle fiber whose myofibrils and sarcosomes can be easily identified. 10 000 $\times$

composed of a single endothelial cell. Although this type of structure is seen in some pictures, one frequently finds that the section has cut across two, or even three, endothelial cells. Our diagrammatic Figure 73 illustrates the possible resultant configurations when a capillary constructed in the manner described above is sectioned in a plane perpendicular to its long axis. As the schematic diagram shows the section can also contain either one or more nuclei. In this connection, it should be kept in mind that these endothelial cells sometimes possess more than one nucleus (Benninghoff 1930). It is, of course also conceivable that the ultra thin section cuts through no nucleus at all. This is illustrated in several of our pictures (Figs 75 and 80). If in such a section demonstrating no nucleus, one finds an area in which the capillary wall seems unusually thick (see Fig 75), perhaps even containing a few plasmasomes one can assume that the section passed in close proximity to the nucleus bringing into the section the mytoplasmic cushion in which the nucleus had resided, but missing the nucleus itself. Generally, the nuclei bulge the membrane interna in toward the lumen of the capillary. The nuclei can occasionally also push the capillary wall toward the outside. As our schematic diagram (Fig 73) illustrates the capillary wall can exhibit one or more mural slits depending on the plane of sectioning. It is only rarely that one sees no mural slits at all and when this occurs it is more probably the fault of a poorly focused photograph than the result of the absence of a mural slit.

The above described ultramicroscopic structure of the capillary wall is found only in the simplest unicellular capillaries the proto capillaries and is a hallmark of this category of vessel. The next step in the development of the capillary towards the pre capillary or post capillary is its widening to the mosaic like arrangement of endothelial cells around the blood stream. There is further on an addition of certain cells surrounding the capillary tube which are collectively termed pericytes Rouget cells or satellite cells (Rouget 1873 1879). These cells are closely applied to the outer surface of the capillary wall. Their nucleus in cross section is sometimes seen to sit like a cap over the nucleus of the endothelial cell (see Kisch 1956b Figs 5 and 6). The protoplasm of the pericyte surrounds the capillary like a hood without enclosing it.



Figure 80 Capillary from the human ventricle between two muscle fibers and an interstitial cell with big nucleus (N) V endothelial villi A Two nerve axons in contact with the capillary SL Sarcolem of the muscle fiber SS Sarcosomes The thick part of the endothelial cytoplasm is a part near to its nucleus

completely, and nowhere does the protoplasm of the pericyte intermingle with that of the endothelial cells. Our pictures illustrate various sections which show how the endothelial cell of the capillary is surrounded by one or more Rouget cells in whose protoplasm one can repeatedly identify nucleus and plasmisomes. By means of the electron microscope, we can differentiate the membrane external of the capillary clearly from the cell wall of the pericyte, a statement which was understandably not so easy for light microscopy. Several pericytes occasionally overlap one another, or surround the capillary from different directions their protoplasm enclosing the capillary like a sheath of surrounding tissue (Figs 79 and 80).

In the part of the vascular system which is known as the precapillary and postcapillary vessels, the diameter of the tube increases in size, and the endothelial layer of these vessels consists then, in each section not of one or two single endothelial cells, but of a larger number. As a rule the typical picture of the protocapillaries then disappears. The individual endothelial cells are no longer flat thin layers of cytoplasm but the cytoplasm tightly surrounds the endothelial nucleus in globular form. In this way the endothelial layer of such small vessels presents the picture known from light microscopy as being a mosaic of endothelial cells surrounded by layers of other cells and finally, further away from the protocapillaries also surrounded by smooth muscle cells.

It should be emphasized that in these pre and post capillaries I also have never seen any material intercalated between the individual endothelial cells which could be interpreted as the cement familiar to light microscopists. I have emphasized for many years that *I strongly doubt the existence of such a cement* which may either be an optical artifact in light microscopy or a precipitation due to the processing of the material for light microscopic investigation.

The result of our own investigation of the vessels concerning the border lines of endothelial cells must therefore be: *a connecting cement between endothelial cells does not exist in the capillaries, nor in other vessels investigated by us to date.* The opinion has been differently proffered that in scurvy the bleeding into the tissue is due to any abnormality of the connecting cement between



Figure 82 Capillary from the leg muscle of an albino mouse. Two endothelial nuclei are visible as are endothelial cell extensions. The capillary is surrounded by two Rouget cells whose plasmosomes are visible. 11 000x.

such experimentations are at present in progress. It is tempting to suppose that red or white blood cells in diapedesis do not figuratively speaking drive with their heads into the wall when an open door is available to them. Of course however such speculations are of little value without the microscopic proof to support them.

Ever since Rouget discovered the pericytes the literature concerning these cells and their physiological significance especially

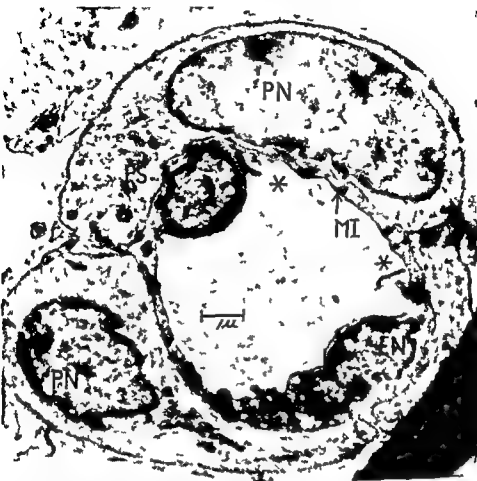


Figure 81 Capillary from a leg muscle of the same mouse like Fig 82. Most probably another section through the same capillary as that illustrated in Figure 82. The nuclei of the pericytes (PN) surrounding the capillary are clearly visible in this picture.  $\times 10,000$ .

the endothelial cells of the capillaries giving the blood cells free space to leave the intracapillary blood stream. In our opinion such a theory must also be rejected first because even normally no such cement exists second because this author in studying the vessels of animals with severe scurvy electron microscopically has never found conditions that would justify such an assumption.

It is at present impossible to say what physiological and pathological significance the mural slits of the capillary may have. Further experimentation will be required to determine this and



Figure 84 A totally collapsed capillary. Within the capillary wall various plasmasomes and extremely fine canals sectioned longitudinally and transversely are visible. Note an elongated thread like endothelial extension in the lumen of the lower portion of the capillary (†). The illustrated capillary is from the left ventricle of guinea pig heart. 22,000X.





FIGURE 83 Totally collapsed capillary from the heart of a guinea pig. Within the lumen whose diameter at the widest part is approximately one half a micron many elongated endothelial cell extensions are visible.  
20 000×

their possible connection with vascular contractility has been extensive indeed (see Krogh 1936). At present our pictures do not permit any conclusions on this point. The obvious folding of the membrana externa in our Figures 81 and 82 would of course, be compatible with a theory that the state of tension or contraction of the pericytes seen surrounding these vessels is the causative factor of the observed state of the capillary wall.

## B THE PICTURE OF THE TOTALLY COLLAPSED CAPILLARY

Up to this point the capillaries which we have shown in our illustrations have all been more or less filled by material in their lumen although these capillaries under the light microscope might well have appeared as collapsed. Some of our capillaries even contained red or white blood corpuscles. The filiform extensions which we noted running from the membrana interna into the lumen of the capillary give an appearance of being suspended in a fluid medium. In cross section these extensions often had a circular appearance (see Figs 75-77-79).

The picture of the totally collapsed capillary is very different. Here the membrana interna in longitudinal sections is separated

## C ABNORMAL CAPILLARIES

It was mentioned above that the probably abnormal configuration of the capillary wall under pathological conditions may be of high and as yet not appreciated importance. The exchange of material between blood stream and tissue cells is thus affected and consequently tissue metabolism at large may be vitally affected as well. In this regard due to the severe handicap of our work as mentioned in the introduction our own experiences are still very limited and mainly involve two pathological conditions. One concerns acute pulmonary edema which we have produced in rabbits by intravenous injection of suprarenin. The animals were killed when in dying condition with pulmonary edema fully developed or when the symptoms of pulmonary edema had just started to develop. The capillaries of the lungs were then investigated (1958a).

As our pictures show our main findings were that many of these capillaries showed a thickening of the endothelial as well as of the epithelial cytoplasm. The endothelium became swollen contained vacuoles and displayed large blisters. Finally it dissolved entirely. Similar changes were found in the epithelial layer of the capillaries of the lung.

These findings correspond closely to those of Meessen and Schulz (1959) based on other experimental methods and published shortly prior to our own findings. In acute pulmonary edema as shown by us (1958a) the endothelial cells in the smallest airducts of the lung produce abundant droplets which must also contribute to fill these air spaces with fluid. These findings lead to considerations as to whether the capillaries of the lung are really the only capillaries suffering under conditions leading to acute pulmonary edema. Indeed this does not seem to be the case. Under these conditions for example we found a thickening of the endothelial wall in some of the capillaries of the heart and we have noticed similar changes in scorbutic guinea pigs in experiments performed together with Dr G J Martin. I think that in the future the concept of a unified pathological condition a generalized capillary edema should be kept in mind if it can be verified by further experimentation.

from the internal boundary of the opposite side by only the finest separation, measuring approximately 500 to 5 000 Å. Sometimes the membrane interna of one side actually touches the membrane interna of the other side however it is always possible to recognize the existence of two distinct membrane layers in such a case. The scalloped nature of the surface is, in general, not obliterated, and the very fine tentacles noted above, whose diameter is approximately 300 to 800 Å do not disappear, but are found to be neatly stretched out and packed into the narrow space which represents the collapsed lumen of the capillary. All this is illustrated clearly in our Figures 83 and 84 which show collapsed capillaries from the guinea pig heart (left ventricle).

If a cross section through a totally collapsed capillary passes directly through an endothelial nucleus the picture may be somewhat different. Here is elsewhere the slit which represents the lumen of the capillary is ultramicroscopically small. Despite this however one can easily see that the nucleus projects far into the lumen of the capillary so that the tiny space which represents the interior of the capillary comes to assume the form of a Y or of a tiny slit. Seeing such pictures one can conceive that in the case of total collapse of a capillary the nucleus projecting as it does into the lumen contributes to the total obliteration of the space.

In sections passing through the long axis of a capillary or in diagonal sections one can see that total collapse such as described above may occasionally occur in only a portion of the vessel so that a totally collapsed portion of capillary may be bounded by another portion whose lumen is patent and is seen in some of our sections even contains some blood corpuscles. Thus while a capillary may be totally closed to the blood stream in reality the section of the vessel whose lumen is actually closed to the point of disappearance may be only several microns in length. In adjacent though patent sections of the capillary the blood stream is stopped so that blood corpuscles are in the stagnant liquid.

The author's own research has not yet advanced sufficiently to allow for an exact description of the ultrastructure of the more complicated components of the circulatory system such as pre-capillaries, arterioles etc. It is our hope however that in the not too distant future we will be able to discuss these subjects as well.



Figure 80 Endothelium from the atrium of a normal guinea pig. A big nucleus (N) in the endothelial cell is seen. In the sub-endothelial stratum which separates the endothelial cells from the sarcolem (SL) a nerve axon (A) 18 000 $\times$

Other structures of the heart richly supplied with nerves are the capillaries both of the atria and of the ventricles. The nerves here are again single axons which make direct contact with the capillary wall. Rarely two or more such single axons are found close to each other and in contact with the capillary wall. Their diameter is between 0.5 and 2 microns. They have a membrane of circular or elliptic shape and sometimes produce a tapering extension along the capillary wall. They contain as a rule cylindrical

## XVII

# INVESTIGATIONS ON CARDIAC NERVES AND THE MECHANISM OF PAIN IN ANGINA PECTORIS\*

Light microscopy and physiology have provided us with many basic facts concerning the cardiac nerves. It is common knowledge that the heart is well supplied with nerves of the autonomic system (sympathic and parasympathic nerves). In certain parts of the heart such as the bundle of His or the sinus node, this has been demonstrated best. It is also known that centripetal nerves are involved in reflexes of the heart such as the Bainbridge reflex or the Bezold-Jarisch reflex. Centripetal nerves of the heart must also be involved in the painful sensation of angina pectoris.

Previously (1956, 1957b) attention was called to single rions which can be found to exist in great numbers in a homogenous subendothelial stratum that spreads between the endocardial cells and the sarcoplasm of neighboring muscle fibers in the auricles as well as in the ventricles. Most of these rions are 0.3 to 0.5  $\mu$  in diameter and are surrounded by a membrane. They contain one or more oblong bodies of cylindrical form up to 2000 Å in diameter and also little dots and fibrils 200 Å or less in diameter which represent internal structures. To date we have found no nerves between the endothelial cells proper.

The existence of the subendothelial stratum and its content of rions has recently been confirmed for the atrium of the turtle as well (Fawcett and Selby, 1955).

\* Part of this and the following Chapter of the book and the different pictures used therein have previously been published in the journal *Experimental Medicine and Surgery*. They are reproduced here with the kind permission of its publisher Brooklyn Medical Press, New York.



Figure 87 Guinea pig Part of a capillary wall (C) with an adjacent nerve axon (A) which contains some big osmiophilic particles. Two of them exhibit an inner lamellar structure. Different small vesicles are seen in the axon (endoplasmic reticulum or droplets). (R) Red cells. 40 000 $\times$

bodies and occasionally finest fibrils similar to those seen in the axons of the subendothelial stratum of the heart. The bigger (1000 to 2000 Å diameter) bodies are probably plasmasomes (mitochondria). Some of them even have a visible internal lamellar structure. The fact that in ultra thin sections of the heart many of the capillaries exhibit nerves at their periphery is proof of the enormous number of axons present at the capillaries of this organ. This constitutes a very definite difference between the capillaries of the heart and those of the lungs which have been examined previously.

Besides the numerous axons attached directly to the capillaries there are also other axons of different diameters occurring singly

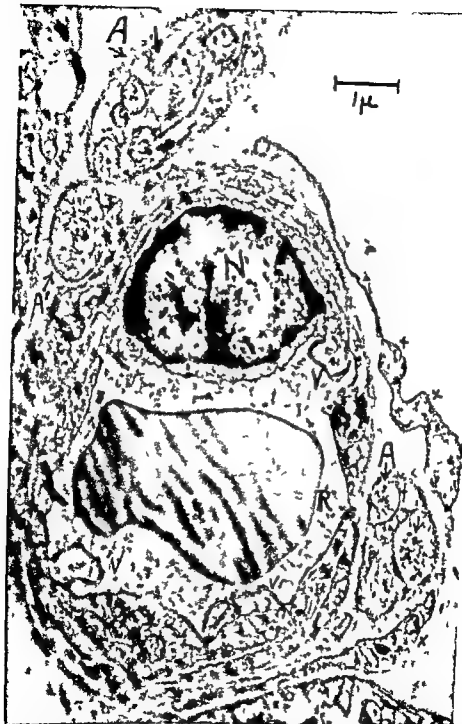


Figure 86 Guinea pig, Capillary in the left ventricle (N) Endothelial nucleus (V) endothelial villi (A) two groups of axons and a single one (A) Notice the difference in the diameter of the individual axons Arrows indicate very fine fibrils in the protoaxons some sectioned longitudinally some transverse (R) Red blood cell 15 000x

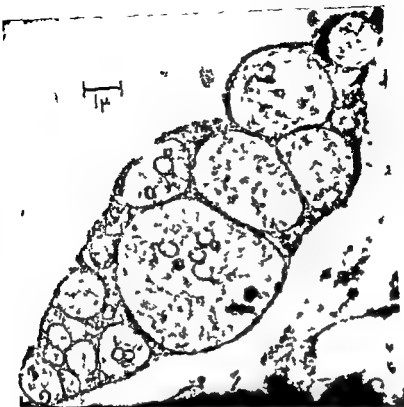


Figure 89 Small nerve fibers from the left ventricle of a normal rabbit. 18 cross sectioned protoaxons are surrounded by a Schwann cell sheath. Notice the very different diameter of the axons. They contain osmiophilic bodies and small vesicles. Between the axons is some cytoplasmic material consisting of lighter and darker droplets and vesicles. 10,000X.

suggests two conclusions: the one concerns the sensibility of the capillaries of the heart in contrast to those of other organs, especially the lungs, while the other concerns the structure of small nerve fibers and consecutive physiologic problems. (See the next chapter.)

Of course the lungs like the heart are well supplied with nerves. However the capillaries of the lungs are not supplied with nerves, whereas as our pictures show there is an abundant amount of nerves in close proximity to the capillaries in the atria as well as in the ventricles of the heart, many of them in direct



and in bundles. They can be found near the pericytes (Rouget cells) and between the individual muscle fibers of the heart. They probably supply both the Rouget cells and the fibers, but they have never been found inside the muscle fibers between the myofibrils. No specific nerve endings at the muscle fibers of the heart have yet been found. See Fig 88 and Chapter XII.

The most outstanding feature of these groups of axons found near the capillaries and between the muscle fibers of the heart is the fact that the axons are very different both in diameter and in their contents. Some of them show the above mentioned big cylindric enclosures and others show instead very thin fibers which appear in cross sections as little dots and in oblique sections as very fine parallel lines. Some axons show both of these structures. From these groups which are surrounded by the sheath of a Schwann cell the single axons apparently separate to reach their final destination, the capillaries or the muscle fibers.

The internal structure and size (0.5 to 2  $\mu$  diameter) of the individual axons in the sub endothelial stratum is very similar to that of most of the axons in contact with the capillaries. We have as yet no means whereby to differentiate the different nerve axons (afferent and efferent) according to their appearance. All the axons found in the heart so far are of the non myelinated type.

A clue to differentiating nerves may be given by the fact that the axons in the sub endothelial stratum are all of a similar type, the diameter being 0.5 to 2  $\mu$  and that they usually contain cylindric bodies (or circular cross sections of them) with a diameter of ca. 1500 to 2000 Å. They also contain fine fibrils with a diameter of 200 Å or less. The endothelium of the heart is not to our knowledge supplied by centrifugal nerves. However influences on the endothelium are known to start reflexes such as those described by Francois Frank, Bezold, Järisch and others. This proves the presence of centripetal nerve fibers. Probably the fibers are those found in the sub endothelial stratum.

The capillaries are reached by single naked axons which are in direct contact with the capillary wall as can be seen from our pictures. They sometimes show a tail like extension along the capillary wall. These contacting axons are of a type very similar to that found in the sub endothelial stratum. This observation



Figure 88 Small nerve fibril from the left ventricle of a normal rabbit. 18 cross-sectioned protoaxons are surrounded by a Schwann's sheath. Notice the very different diameter of the axons. They contain osmiophilic bodies and small vesicles. Between the axons is some cytoplasmic material consisting of lighter and darker droplets and vesicles. 10 000X.

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contact with the capillary wall. It seems very probable that this fact is responsible for the intense pain in conditions of inadequate blood supply to the heart as for instance in attacks of coronary spasm (leading to the pain called angina pectoris) or after occlusion, even of a relatively small coronary vessel. By contrast major destructions of the lungs even such secondary to inadequate blood supply may occur without any associated acute pain.

Concerning the internal structure of the small nerve fibers in the heart, our pictures show that nerve bundles of a diameter of only a few microns, scarcely visible to light microscopy contain many, distinctly separated individual axons of variable diameter. Such entire groups being bundles of protoaxons are usually enveloped by the sheath of a Schwann cell. From this observation it can be concluded that in separating from a nerve fibril, the finest axons do not follow a pattern of arborization in which one bigger axon fiber splits in two smaller branches. Rather, within any nerve fiber there are many individual axons (proto axons), all separated from one another (1955c). These axons probably run uninterrupted from the central cell to the periphery. Their

branching from the nerve fiber is in reality only a separating of individual units from each other. It is not a division of one common fiber. I also have never seen the dividing of one axon into two in diagonally sectioned axons or in longitudinal sections. The recognition of this structure of the nerve fibers creates new problems for the physiologist and for the pathologist too. For instance the usually accepted concept of the nonreflexes is incompatible with these histological facts. (See Fig 85 and next chapter.)

The individual most primitive axons or proto axons have a very different diameter. Various reasons may be responsible for such a phenomenon. Perhaps within a nerve fibril, axons of different diameter and cylindrical appearance exist next to each other as was proven to be the case by Tisaki and his collaborators (1944) and G. Rudolph (1956) in myelinated nerves. Recently similar observations were made in the atrium of the turtle by Fawcett and Selby (1958). The single axon is not cylindrical but changes its width irregularly according to its content of plasmisomes (mitochondria) (Kisch 1958c). (See Fig 86 and Chapter XIX.)

It may also be that the appearance of the cross sections of axons in the heart should be attributed to the presence of different kinds of nerves for instance centripetal ones and centrifugal ones. In this case I would be inclined for reasons mentioned above to regard the big axons with cylindrical and spherical osmiophilic inclusions as centripetal nerves. Further investigations will be needed to clarify this point.

The clinical syndrome of angina pectoris is well known for a long time. Our knowledge concerning the mechanism of the severe pain observed in acute ischemia of the heart muscle is very vague however. This ischemic pain is present in any type of coronary insufficiency, in coronary occlusion in attacks of severe tachycardia and in tight aortic stenosis. The usual explanation that sensory nerves become irritated under such conditions and cause pain is of course not satisfactory because sensory nerves are irritated in any kind of pain. We know for example that similar deficiency of blood supply to a portion of the lungs does not produce similar intense pain despite the fact that the lungs too are well supplied with nerves. Even the pain following multiple embolism to the lungs is hardly comparable in intensity to the pain of angina pectoris.

The extremely rich nerve supply of the capillaries in the human heart has been noticed (1958c) and some evidence has been offered that these capillaries are supplied by centripetal and centrifugal nerve axons mainly by the former. In electron microscopic studies on the ultrastructure of the circulatory system (1957g) ultra thin sections of the heart muscle (atria as well as ventricles) very frequently showed nerve axons in close proximity to or in direct contact with the capillary wall. Considering this fact one can visualize the incredibly rich nerve supply of these capillaries. By contrast this author's own previous investigations of the lungs and their capillaries established the fact that the capillaries of the lungs have no nerve axons in contact with them.

These observations lead one to the conclusion that of the many nerves which supply the lungs as well as the heart those in contact with the capillaries are chiefly responsible for the pain of angina pectoris. In spasm or occlusion of a coronary artery sudden chemical and physico chemical changes of the blood in the capillaries

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## XVIII

### THE MECHANISM OF AXON REFLEXES

The experience which this author has gained in investigating the nerves of the heart by means of electron microscopy have led him to certain conclusions concerning axon reflexes. These will be proffered here because a great deal of the literature on axon reflexes concerns the cardiovascular system. This is true for the investigations of Nimian Bruce (1913) on vasodilator axon reflexes and for the investigations on similar reflexes reported by A. Krogh, Sir Thomas Lewis and others. Because recently there has been doubt as to the existence of underlying axon reflexes for some of these observed reflex reactions (Schaefer 1952) it seems necessary here to confront the physiological aspects with the histological facts.

According to the 21st Edition of the *American Illustrated Medical Dictionary* (1948) an axon reflex is "a reflex resulting from a stimulus applied to one branch of a nerve which sets up an impulse that moves centrally to the point of division of the nerve where it is reflected down the other branch to the effector organ."

This definition expresses well what one usually has in mind when speaking about axon reflexes.

It was probably the physiologist and biochemist Willie Kuhne who in 1859 first described an axon reflex fitting the above mentioned definition though he did not yet use the expression "axon reflex." At the centenary of Kuhne's discovery of 1859 it seems appropriate to check and revise our ideas concerning the mechanism of axon reflexes in the light of electronmicroscopic experiences.

Kuhne's basic experiment, his famous *Zwei Zäpfel Versuch* (two tips experiment) was performed with the musculus sartorius of the frog.

may be the irritant which stimulates the sensory nerves of these capillaries. Similar changes in the immediate surroundings of these capillaries due to an abnormal circulation may have the same effect on the centripetal nerves in contact with the capillaries. Such a concept could for instance explain the following well known fact. In an anoxemia test, the lungs as well as the heart are insufficiently supplied with oxygen. There is no pain in the lungs, even in high degrees of anoxemia but there is pressure and pain precordially especially when a coronary insufficiency is present. This pain is also seen without a coronary involvement in cases of generalized anoxemia of the heart is, for instance in a severe tachycardia or in aortic stenosis. We also know from experiments of E. Zalk that if a tourniquet is put around one arm of a healthy person and he then does the same amount of exercise with both arms, he will soon feel severe pain in the arm with insufficient blood supply. The pain experienced by patients with intermittent claudication is also well known. Electron microscopic studies of skeletal muscle reveal that indeed the capillaries of this type of tissue also are well supplied with nerve axons (our observations).

This author's findings concerning the abundant nerve supply to the capillaries of the heart (1957g see Figs 80 and 89) have recently been corroborated by Meessen (1959). Axons adjacent to muscle fibers of the ventricle are much rarer but they too have been observed (Kisch not yet published).

with some also on the pilomotor of the dog and on vasomotor reflexes of the skin of both these animals. His conclusion was that his observations may also be applied to all classes of efferent nerves existing in the sympathetic system (p. 364).

A. Nimian Bruce reported in 1913 on *Vaso Dilator Axon Reflexes* summarizing in the introduction all the previous literature on the topic.

Axon reflexes especially some involving the vessels of the skin were reported or at least suggested by many authors. Among these were Th. Lewis, Krogh, L. H. Muller, Ebbecke, Fleisch, R. Tigerstedt summarized most of the pertaining work up to 1923 in his classic *Physiology of Circulation*. Recently however Hans Schaefer (1952) has uttered some doubt concerning the real existence of some of the axon reflexes described in the literature.

Not many of the authors reporting axon reflexes have cared to give their opinion on the finer mechanism of such a reflex. Most of these if not all seem to have in mind a mechanism as suggested by Wilhe. Kühne in Figure 90. We should like to call this the classic concept of axon reflexes. A single nerve fiber divides itself into two branches. Within this unit the impulse travels up one branch (centripetally) to the point of division and from there down the other branch (centrifugally) and probably also up the undivided fiber. Nimian Bruce expresses his idea of the mechanism of an axon reflex as follows (p. 351):

"When the fiber comes near to the periphery it must bifurcate like the letter Y, one limb passing to the sensory end organs etc. in the skin and along this afferent impulses will pass while the other will end in the bloodvessels and along this efferent impulses will pass."

Such a concept is made of course under the never proven assumption that a primitive nerve fiber is able to bifurcate in the periphery like the letter Y. It seems that nobody ever has had a serious doubt about this prerequisite of the classical concept of axon reflexes.

In studying for many years the electron microscopy of the constituents of the circulatory system I have recently mainly investigated the cardiac nerves. The result of these studies gave reason





Figure 89 Ventricle of the heart of the rat. Next to capillary C with endothelium Nucleus N in a fine nerve fiber cross sectioned containing two protofibrils. 9000X

Our Figure 90 is a reproduction of Figure 9 in Kuhne's book of 1860 (p. 98). The sartorius is cut at its tip with scissors to a point where there is finally in upper common part of the muscle and two separate tips (A and B) as in our Figure 90. A as well as B are supplied by one branch respectively of the nerve N which as can be seen, divides itself at point t into two branches. These branches do not reach further than to line M. The muscle beneath this line is bare of any nerve supply. That just makes the frog's sartorius fitted for Kuhne's experiment. He observed that chemical or mechanical stimuli applied to the nerveless part of tip B, first at the level of a a a produce a contraction of the fiber B only. A remains at rest. If a stimulus is applied to the branch of the nerve in B at the level of line b (Fig. 90) both tips of the muscle (A and B) contract. Kuhne offered for this phenomenon the explanation which remained until today the concept of anaxon reflex. This is indicated by arrows in his figure. The impulse produced by the stimulus in b goes according to Kuhne centripetally within the nerve up to point t and from there centrifugally down into branch A. Kuhne regarded this experiment as a convincing proof of the ability of motor nerve fibers to conduct impulses in both directions (*Doppelsinniges Leitungsvermögen der motorischen Nerven*).

It was in 1900 that J. N. Langley described exactly the same process for preganglionic fibers of the sympathetic system without reference to Kuhne's experiment. Langley created for these pseudoreflexes the name anaxon reflex. This name has been generally accepted ever since in neurology. Langley's experiments were mainly performed on the pilomotor nerves of the cat.

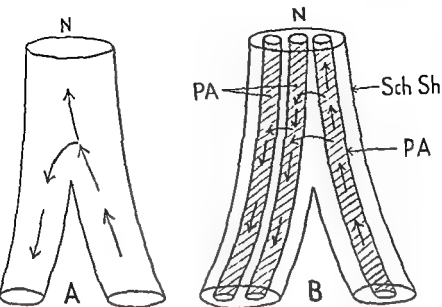


Figure 91: Diagram of an axon reflex. A The classical concept. B The new concept based on the electron microscopic pictures. See text.

tions are in best agreement with pictures of nerve tissue presented by other electronmicroscopists as for instance the fine work of H. Gansler and others. These authors however abstained from discussing the ultramicroscopic facts in regard to the branching of nerve fibers or the important repercussions of the electronmicroscopic findings on physiological problems.

Our Figure 91 shows in form of diagrams in A. Kuhne's concept of axon reflexes and in B the histological facts and the only possible mechanism for axon reflexes if such reflexes really exist.

It seems remarkable indeed that the very same problems which we have just emphasized with regard to the electronmicroscopic structure of protoaxons and the dividing of nerve fibers were discussed already more than a hundred years ago. At that time the discussion involved the smallest nerve fibers visible to the naked eye or those visible through the very primitive microscopes of this time. Reil then regarded the thinnest nerve fibers to have a diameter of a hair of the scalp whereas other scientists thought such a guess to be wrong (H. E. Weber 1830). They supposed

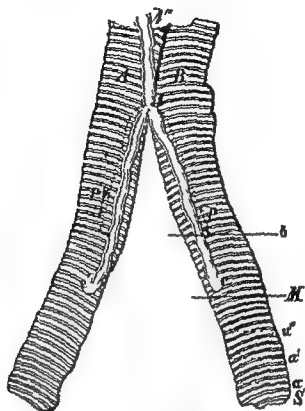


Figure 90. *Wilhe Lubmes (1859)* drawing to explain the mechanism of his two tips experiment. See explanation in the text.

seriously to doubt whether branching and dividing of an individual most primitive nerve fiber really exists. I was able to prove for the unmyelinated nerve fibers of the heart and in not yet published investigations for the myelinated nerve fibers of the leg muscles the following fact. The smallest nerve fibers exist either as single axons (diameter 0.3 to ca. 3  $\mu$ ) or as bundles of such protoaxons surrounded by a Schwann sheath. In diagonal or longitudinal sections I have not yet seen the dividing of a protoaxon. I came to the conclusion that according to electron microscopic evidence all branching of the finest nerve fibers is only a separating of preformed individual protoaxons or groups of protoaxons, never a dividing of a common stem similar to the stem of a tree dividing into branches.

If this new aspect proves to be correct, the classic concept of axon reflexes is untenable. Our own electronmicroscopic observa-

## XIX

### SOME PICTURES FROM THE HUMAN HEART

The material for the following pictures was taken from patients who died in a condition of chronic heart failure. Particles from the ventricle of the heart were taken immediately after the death of the patient by needle biopsy method by Dr Mehmed Cavusoglu of the medical division of City Hospital at Elmhurst New York.

Special attention is called to the many sarcosomes with disintegrated internal structure (osmiophilic degeneration) in these hearts. Such sarcosomes are not found in the hearts of normal animals. (See Kisch Cavusoglu and Maringoni 1959)

that the diameter of the smallest nerve fibers is one quarter of a hair diameter only. Robert Remak reports a remark of his master Johannes Muller, that probably the so called finest nerve fibers are by no means really the finest ones and that the really finest ones are surely not yet known at that time (ca 1835). In this respect a remark of the great anatomist and physiologist H. E. Weber (1830) about ramification of nerves made in 1830 is noteworthy. In literal translation it reads on page 277 of his book: Most nerves divide approximately like a tree into smaller and still smaller branches but in most cases one can prove that all these branches starting from one nerve consist of fascicles of nerves or nerve fibers which already before the division were isolated within the stem and have had their own individual enwrapping, wherever this can not be proved the reason is the smallness of the branches. However it has never yet been observed by anyone that a simple nerve fiber has divided into branches.

This statement of Weber holds according to our experience for the protoaxons too. They are probably or better surely just those finest nerve fibers (diameter from up to  $0.3 \mu$ ) of which Johannes Muller and Robert Remak dreamed 120 years ago (Kisch 1954).

As mentioned above the physiological consequences of this recognition make it necessary to abolish the classical concept of the mechanism of axon reflexes as conceived by Kühne (Fig. 91A). If axon reflexes really do exist their mechanism ought to work according to the diagram in Figure 91B. That of course creates a new very great difficulty: an impulse ought to be transmitted from one protoaxon across its own axon membrane transversally to a neighboring one across the membrane of this second protoaxon and through the matrix that separates the protoaxons. A biochemical stimulus transmission seems to fit such a setup much better than an electric transmission.

That means that in case of axon reflexes we would have to assume that at least all the protoaxons enwrapped by the same Schwann's sheath are only anatomically but not functionally units isolated from each other.

Such an aspect of course calls for a host of new experiments in the field of neurophysiology and applied biochemistry.



Figure 93 : Another picture of the human ventricle. Notice the large amount of sarcosomes between the double lined sarcolem (SI) and the myofibrils and between the individual myofibrils. Some of the sarcosomes show an inner lamellar structure the content of others is retracted and disorganized.  
14 000x

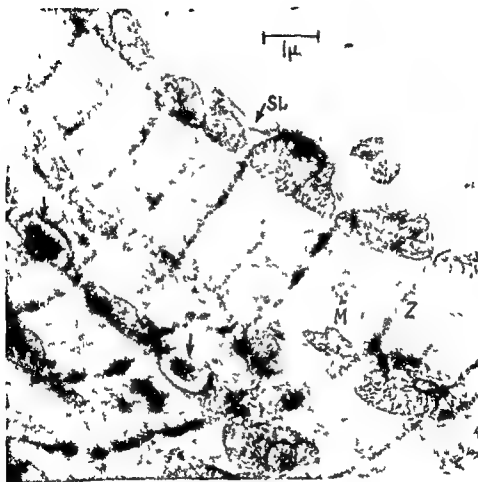


Figure 92 Myofibrils with typical striation. The zones Z and M are clearly visible. sarcosomes with an inner lamellar structure fill the space between the sarcolem (SL) and the myofibrils and the space between the individual myofibrils. Two sarcosomes indicated by arrows show osmiophilic degeneration. Their membrane is still present but their content is retracted and dark. Length of each sarcomere (the distance between two Z lines) is about 2 microns. Human ventricle. 12 000×



Figure 93. Arrows point to an intercalated disc in the human heart. A double lined zig zag membrane can be seen. The intercalated discs are regarded by most authors as boundary lines of the muscle cells of the heart. Notice the many sarcosomes (SS) some of which are in osmolytic degeneration. 17 000x





Figure 94 Sarcosomes of the human heart. Some with normal inner structures, two in osmiophilic degeneration. Sarcosome indicated by arrow is very big and its content is a more or less homogenous material. Next to it indicated by asterisk is osmiophilic body. 12 000 $\times$

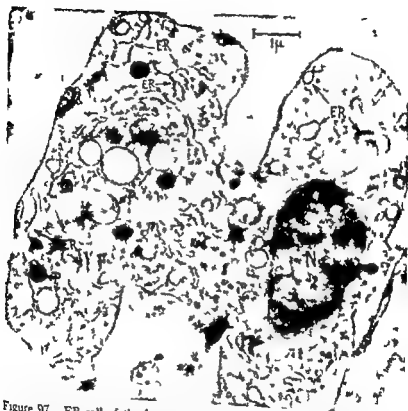


Figure 97 ER-cell of the human heart. Usually located near a capillary but also found between muscle fibres. Characterized mainly by the widened endoplasmic reticulum (ER) spaces by many ghosts of plasmalomes (P) (mitochondria) and by many small granules in the cytoplasm. The nucleus (N) is usually eccentric. These cells are reminiscent of certain types of plasma cells (Kisch 1960) 12 000X

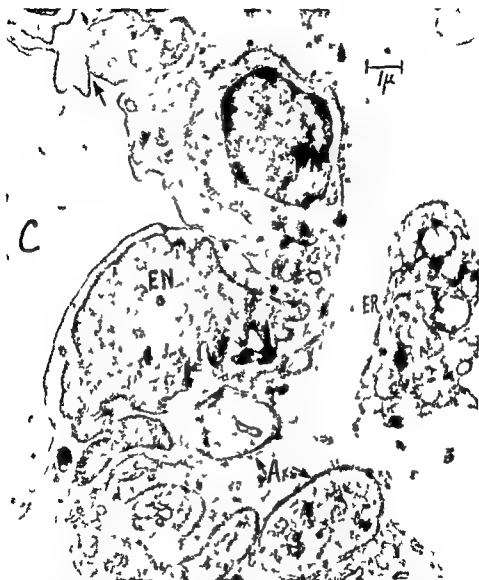


Figure 96 A capillary wall from the human heart. C indicates the lumen of the capillary. EN = nucleus of bizarre form of an endothelial cell. In the cytoplasm of the cell are mitochondria and smallest droplets. Attached to the external membrane of the capillary is a Pericyte. PN = nucleus of the Pericyte. Ax = nerve axons. RE = part of an RE cell (see next pictures). Arrow points to slit in the capillary wall. 8000×

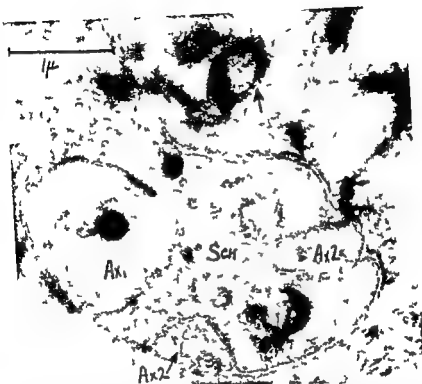


Figure 99 Section of a small nerve fibre between muscle fibres. Three axons (Ax) are surrounded by a Schwann (Sch) cell. Notice the difference between the diameter of the big axon Ax1 and that of two small axons Ax2. They probably represent two different types of nerves. It seems that the axons are free of the Schwann cell at one side. The body indicated by arrow may be a naked axon. Mitochondria can be seen in the Schwann cell as well as in the axons and also in the smallest vesicles. Outside of the axon finest fibrils probably collagen fibres can be seen. 31 000x

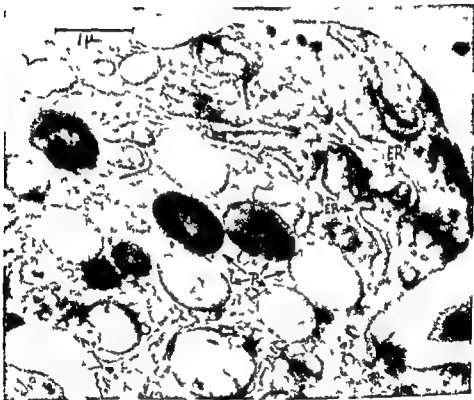


Figure 98 Enlarged part of an ER cell of the human heart. Plasmisomes (mitochondria) with their dark content can be seen. There are also ghosts of plasmisomes (P). Small granules are attached to the widened channels of endoplasmic reticulum (Palade granules). The same granules are seen everywhere in the cytoplasm. ER = endoplasmic reticulum. 17 000×

3 Muscle fibrils of cardiac muscle and the fibrils of other types of striated muscle exhibit no basic differences. The possible exception to this might be the Z bands which connect the individual muscle fibrils of the myocardium but not those of Purkinje's fibers.

4 Electron microscopic studies of the capillaries and of the immediately adjoining Rouget cells have revealed several thus far unknown facts. For example, it was found that the endothelial cells of the capillary contained plasmisomes and an endoplasmic reticulum. The capillary wall contained even in protocapillaries always one or more mural slits. These slits do not occur only in places where two separate endothelial cells touch but rather they are also found to occur where the margins of a single endothelial cell, surrounding the lumen of the tiniest capillary (protocapillary) overlap. We could never discover any special substance (cement) occurring within the capillary wall at the endothelial cell boundaries or in the endothelial slits.

Physiologically, it is especially noteworthy that the inner surface of the capillary is scalloped and that long ribbon-like extensions from the endothelial cells may stretch deep into the blood stream within the capillary lumen (endothelial villi or tentacles).

5 The cardiac nerves have been studied and their location and ultramicroscopic structure suggested certain concepts about the mechanism of the pain in Angina pectoris and the mechanism of axon reflexes. Axon endings at muscle fibers of the human ventricle have been found for the first time.

6 The results of the electron microscopic investigations of the conductive system (Purkinje's fibers) seem noteworthy as the first of this kind.

7 The mature red blood cells in mammals are the only cells of the body containing no plasmisomes (mitochondria) and no endoplasmic reticulum.

8 In chronic heart failure in man a progressive osmiophilic degeneration of sarcosomes has been found and linked to functional disturbances and to the presence of lipofuscin and other lipids in muscle fibers of such hearts.

9 Droplets of ultramicroscopic size have been found in the capillary wall in muscle fibers and in nerve axons. They are supposed to present an important type of material exchange in

## FINAL REMARKS

Physiologists and cardiologists will always be suspicious upon hearing conclusions concerning the function of organs which are substantiated by histological pictures only. The author himself a physiologist and cardiologist entered upon the extremely difficult field of electron microscopic histology with the greatest of trepidation and only for the express purpose of finding guides, in the ultramicroscopic structure of the heart towards the solution of certain unsolved problems in cardiology. While the results of more than ten years of research under the worst financial conditions are not overwhelming they are still encouraging enough to inspire further work. The following achievements seem, to the author most worthy of note

- 1 The understanding of the function of the sarcosomes and recognition of the importance of their varying quantities in the different types of muscle has provided a first solution to the long standing problem of why the heart can beat throughout a lifetime without requiring an extended period of rest a feat impossible to skeletal muscle. Through further comparative studies, we have come to realize that the ability of a muscle to do heavy steady work can be mathematically stated as a function of the number and type of sarcosomes found within its muscle fibers. This would seem to hold not only for the varying muscle groups within the same animal but also for the same muscle in different species within the animal kingdom (big mammals like cattle small mammals like the rabbit guinea pig rat mouse but cold blooded animals like frog fish)

- 2 Differences in the sarcosomes of the auricle and ventricle of the same heart could be determined

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living cells and create many new problems in regard to droplet transfer versus molecular transfer of material in living cells

Many items mentioned in the various chapters and demonstrated in the figures have a physiological meaning which is as yet not elucidated. Such items include the inter fibrillar membranes in the muscle fibers, and also straight parallel and concentric lamellae, and the variations in their appearance in the different sarcomeres. The uncertainty as to physiological function which surrounds these items extends also to the ultramicroscopic canal system, which is found in the cardiac muscle of humans as well as of all other animals investigated to date and in the cytoplasm of the capillary wall (endoplasmic reticulum)

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# INDEX

## A

- Abbe Ernst 3  
 Acetylcholin 82  
 American College of Cardiology ix  
 Amphibia, 25 III  
 Angina pectoris viii 138 ff  
 Anovemia test 148  
 Artifacts viii ix 68  
 Ashoff L. 112  
 Atrium 48 49 58 67 90 91 93 96  
 126  
 Auricle see Atrium  
 Axons 109 117 118 123 131 140 ff  
 148 ff 161  
 Axonreflexes viii 144 147 ff

## B

- Bambridge reflex, 138  
 Baker 28  
 Bamboostick fibrils 10  
 Bat 26 27 89  
 Beams 31  
 Bee see Honey bee  
 Bennet E St 93  
 Bennet and Porter 14 20 24 25  
 Benninghoff A 127  
 Bernstein J 92  
 Bezold Jarisch Reflex 138 142  
 Birds 84  
 Borysko E 8  
 Bornet Bodo von 3 4  
 Bourne C H 76  
 Bowman 12 46  
 Braunsteiner 62  
 Bremen V L van 36 37 94 95  
 Brunner 82  
 Bumble bee 23 24 25 37

## C

- Capillaries vii 13 47 83 104 115 118  
 119 ff 127 131 158

- Capillaries abnormal 137 ff  
 Capillary edema 137  
 Cardiac nerves see Nerves  
 Cement 124 ff 130 132  
 Chalkin 82  
 Chapman 11 20 22 24 25 37  
 Chicken 25 95  
 Claudication intermittent 146  
 Cleland and Slater 76  
 Cohnheim 28  
 Conductive System 112 ff  
 Confetti-cells 105 106  
 Coronary insufficiency 143 146  
 Coronary spasm 144 ff  
 Cow 25 66 68 78 80 91 96 101  
 111 113 ff 117  
 Cross striation macroscopic 28 ff

## D

- Dalton and Felix 91  
 Deems Evans Jauney and Baker 23  
 Diagrams 11 29 41 120  
 Diaphragm 49 50 55 64 78  
 Dobie 28  
 Dog 25 94  
 Draper and Hodge 14 26 31  
 Droplets 12 93 125 127 141 163  
 Droplet dialysis 12 93  
 Droplet transfer 12 93 127 163

## E

- ER Cells 107 159 160  
 Ebbecke U 149  
 Edwards G A 50  
 Eel 84  
 Electrophysiology 92 ff  
 Embryonic heart 36 95 99  
 Endocardium 108 ff  
 Endoplasmic reticulum 12 65 67 79  
 83 ff 89 91 93 117 123  
 Endothelial villi, 122 ff 126 140

Endothelium 107 108 ff  
 Engelmann 29  
 Enzymes 76 77 81 85 90 ff  
 Epithelium 104  
 Ergastoplasma 12 91  
 Erythrocytes *see* Red Blood Cells  
 Eyemuscle 60 78

## F

Farrint and Mercer 36  
 Fawcett and Selby 18 66 133 144  
 Finch 77  
 Fish 12 78 84  
 Flusch 149  
 Frog, 12 25 62 65 78 95 148

## G

Galen 56  
 Gansler H 151  
 Gersh 8  
 Ghosts of sarcomeres 68 72 77  
 Golgi apparatus 91  
 Guinea pig 20 22 23 25 26 30 50  
 51 59-61 69 70 90 95 98 99 100  
 105 109 122 129 134 135 139  
 140 141

## H

Haghaquist 28  
 Hall Jakus and Schmitt 24 26 31  
 Hamster 77  
 Heart failure 77 84 ff 153 ff  
 Heart function *see* 46 ff 84  
 Heart rate 77 78  
 Heidenhain 28  
 Henle 12 46  
 Heinsen 31  
 Hexagonal packing of protofibrils 21  
 22 23 24  
 His W Jr 112  
 His bundle of 112 138  
 Hodge A J 20 22 24 25 26 27 39  
 40 ff  
 Hodge and Chapman 45  
 Hodge Huxley and Sparo 11 20 22  
 23 25 37 40 ff  
 Holmgren 12 47  
 Honey bee 25 37 53

Hornet 37 52  
 Horse fly 37

## I

Insect flight muscle 10 12 20 22 24  
 25 30 42 43 47 50 66 81  
 Intercalated discs 11 94 ff  
 Interstitial cells 104 ff 129

## J

Jordan 28 32

## K

Kisch Bruno von 12 14 18 25 32 37  
 39 46 ff 86 95 96 101 107 121  
 126 128 146 152  
 Kisch and Cavusoglu 84  
 Kisch Cavusoglu and Mirangom 81  
 153  
 Kisch and Philpott 12 21 24 29 30  
 56 62 73 84  
 Koelliker A v 12 28 46 68  
 Krause 28  
 Krogh A 121 125 127 134 147 110  
 Kuhne W 82 147 ff 150 151

## L

Lingky J N 148  
 Linzavacchia 95  
 Lehmann Otto 24 70  
 Lewis Th 147 149  
 Lieegang R E 39 41 42  
 Light microscopy *see* 13 ff  
 Lindner v. de Poche and Linder 18  
 25 32 39 95 97  
 Lipofuscin 85  
 Liquid Crystals 23 ff 70  
 Low F N 104  
 Lungs 104 106 144 ff  
 Lung embolism 145

## M

Man heart of 23 25 31-35 44 48 50  
 67 74 79 84 ff 87 92 95 99 100  
 102 105 123 126 127 131 146  
 155 ff  
 Martin I J v 137  
 McDougal 28  
 Mettly 20 25 37 42 43 71 72

- Mee en and Schulz 137  
 Mee en 146  
 Meis 29 47  
 Melland 28  
 Membranes 86 ff  
 Membrane nuclear 101  
 Metabolism 101  
 Methods vii viii 3ff  
 Mitochondria (see also Plasmavomes)  
   46 47 64 65 67 76 82 84 141  
 Moderstorband 112 ff 118  
 Monckeberg 118  
 Moore and Ruks 20 22 23 32 50  
   97 98  
 Morgan 22  
 Mouse 15 17 26 49 50 54-58 73  
   77 81 83 93 99 132 133  
 Muir 93 97  
 Muller Joh 152  
 Muller L R 149  
 Muscular slits in Capillaries 81 107 122  
   ff 126  
 Musculus pectoralis 31 32 33  
 Musculus psoas 34 35  
 Musculus sartorius 148 ff  
 Muscle fiber structure 10 ff  
 Myofibril 10 19 ff 30  
 Myofibrilament see Protofibrils
- N
- Napolitano and Fawcett 68 72  
 Nerveaxon see Axons  
 Nerves cardiac 13 109 131 138 ff  
   181  
 Nerves endplates 82  
 Ninian Bruce 147 149  
 Nomenclature 46 47  
 Nuclei 99 ff 115 128  
 Nucleoli 100 101 102
- P
- Palade C (see also Porter and P) 7  
   56 68 90 91 118 126  
 Pease and Baker 8 11 18 32 37 39  
 Pericyte (Rouget cells) 104 ff 120  
   ff 128 129 132 ff  
 Philpott D (see also Kisch and Phil  
   pott, 19 5
- Philpott D and A Szent-Gyorgyi 31  
   36 39  
 Typefish 16 39 89 93  
 Plasmavome 107 159 160  
 Plasmavome (see also Mitochondria)  
   47 75 79 80 81 82 ff 126 ff 141  
   144  
 Plant 76  
 Loche u Lindner 18 95 97  
 Polymorphism of sarcomeres 50 66  
 Porter L and Palade C 20 25 50  
 Postcapillaries 130  
 Powers 71  
 Precapillaries 130  
 Protoaxons see Axons  
 Protocapillaries 120 ff  
 Protofibrils (myofibrilament) 19 ff 39  
 Pseudostriation 40 ff  
 Pulmonary edema 137  
 Purkinje J E 112  
 Purkinje fibers 37 92 96 112 ff
- R
- Rabbit 110 116 118 143  
 Ramsay and Warren 82  
 Rat 25 50 63-65 77 148  
 Red blood cells 79 80 82 121  
 Reil J C 151  
 Remak Robert 152  
 Reticulocytes 82  
 Retzius C 12 29 46 47  
 Rhodin J 56 69  
 Rollet 28 36  
 Rouget 128 133  
 Rouget's cells (see also Pericytes) 104  
   120 128 130 132 133 142  
 Rozsa Szent-Gyorgyi and Wyckoff 24  
 Ruska E 3  
 Ruska H 14 117 190
- S
- Sarcolem 10 14 ff 44 45 ff  
 Sarcomere 29 29  
 Sarcomeres viii 12 27 38 39 42 43  
   46 ff 99 102 103 116  
 Sarcomere theory of the heart 40 ff  
 Schaefer H 147 149  
 Schlipfoter vii  
 Schwann-cells 142 143 144



Sea gull 77  
 Sickewitz 70  
 Sinus node 138  
 Sjostrand I S 50 67  
 Sjostrand and Anderson 95  
 Skeletal muscle viii 10 ff  
 Slater and Cleland 76  
 Slit in capillary wall 81 107 122 ff  
     126  
 Sparrow 77  
 Specific cells of the lung vii  
 Stratum subendothelial 138 ff  
 Striation microscopic 28 ff  
 Striation transvers 26 ff  
 Striation ultramicroscopic 26  
 Syncytium 10 97  
 Szent Gyorgyi 39 65

## T

Tachycardia 145 146  
 Tawara 112

Teasing preparation ix 49 119  
 Tentacles *see* Endothelial villi  
 Thrombocyte 75 83 121  
 Tikcrstedt R 149  
 Trichites 70  
 Tsumura N 84  
 Tumorcells 82

## W

Warburg O 82  
 Wasp 20 21 25 37 73  
 Watanabe and Williams 76  
 Watson M L 78  
 Weber H E 151 152  
 Wyckoff 3

## Z

Z-band 28 37 39 44 113  
 Z-Sarcosome 50 79  
 Z Zone 28 39  
 Zack E 146

